

# Diversity of the strongly rheophilous tadpoles of Malagasy tree frogs, genus *Boophis* (Anura, Mantellidae), and identification of new candidate species via larval DNA sequence and morphology

Roger Daniel Randrianiana<sup>1,2</sup>, Axel Strauß<sup>3</sup>, Julian Glos<sup>4</sup>, Miguel Vences<sup>1</sup>

**1** Division of Evolutionary Biology, Zoological Institute, Technical University of Braunschweig, Mendelssohnstr. 4, 38106 Braunschweig, Germany **2** Département de Biologie Animale, B.P 906, Université d'Antananarivo, Antananarivo, Madagascar **3** Institut für Genetik, Ludwig-Maximilians-Universität München, Großhaderner Str. 2-4, 82152 Martinsried, Germany **4** Zoological Institute, University of Hamburg, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

Corresponding author: Roger Daniel Randrianiana (roda.randrianiana@googlemail.com)

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## Abstract

This study provides detailed morphological descriptions of previously unknown tadpoles of the treefrog genus *Boophis* Tschudi and analyses of habitat preferences of several of these tadpoles in Ranomafana National Park. A total of twenty-two tadpoles determined via DNA barcoding are characterized morphologically herein, fourteen of them for the first time. Twelve of these tadpoles belong to taxonomically undescribed candidate species which in several cases are so far only known from their larval stages. Our data show that the larvae of some of these candidate species occur syntopically yet maintaining a clearly correlated genetic and morphological identity, suggesting that they indeed are true biological and evolutionary species. Tadpoles considered to belong to the “adherent” ecomorphological guild inhabit fast-running waters and their oral disc is commonly to continuously attached to the rocky substrate, supposedly to keep their position in the water current. Some of these species are characterized by the presence of a dorsal gap of papillae and the absence of an upper jaw sheath. This guild includes the tadpoles of the *B. albipunctatus* group (*B. ankaratra*, *B. schuboeae*, *B. albipunctatus*, *B. sibilans*, *B. luciae*), and of the *B. mandraka* group (*B. sambirano* and six candidate species related to this species and to *B. mandraka*). Tadpoles considered belonging to the “suctorial” guild inhabit fast-running waters where they use frequently their oral disc to attach to the substrate. They have an enlarged oral disc without any dorsal gap, including two nominal species (*B. marojezensis*, *B. vittatus*), and five candidate species related to *B. marojezensis*. An ecological analysis of the

tadpoles of *B. luciae*, *B. schuboeae* and *B. marojezensis* [Ca51 JQ518198] from Ranomafana National Park did not provide evidence for a clear preference of these tadpoles to the fast flowing microhabitat sections of the stream, although the tadpoles discussed in this study are typically caught in this habitat.

### Keywords

Amphibia, Anura, Mantellidae, *Boophis*, larval morphology, oral disc, clasping, adherent, suctorial, candidate species, larval ecology, microhabitat preference

### Introduction

The genus *Boophis* Tschudi, 1938 is a species-rich group of treefrogs in the family Mantellidae which is endemic to Madagascar and to the Comoran island of Mayotte. Seventy-two nominal species and over 25 candidate species of *Boophis* are currently known (Vieites et al. 2009, Vallan et al. 2010, Glaw et al. 2010, Vences et al. 2010a, b).

Tadpoles have been described for 46 species of *Boophis* (e.g., Blommers-Schlösser 1979, Thomas et al. 2005, Grosjean et al. 2006, Raharivololoniaina et al. 2006, Altig and McDiarmid 2006, Glos et al. 2007, Schmidt et al. 2008, Randrianiana et al. 2009a, b, Rasolonjatovo et al. 2010). Compared to many other Malagasy anuran groups, the larval stages of *Boophis* are therefore quite well known, possibly because they are relatively easy to find in rainforest streams (e.g., Strauß et al. 2010) and even sometimes outside the forest (Glaw and Vences 2007).

The existence of strongly rheophilous tadpoles in species of *Boophis* has been known since the work of Blommers-Schlösser (1979). This author pioneered our understanding of the evolutionary relationships and natural history of Malagasy frogs and described several tadpoles with peculiar morphological characteristics such as an enlarged oral disc, increased number of keratodont rows and papillae, and low tail fin. These larvae were assigned to *Boophis majori*, *B. erythrodactylus*, *B. mandraka*, and *Boophis* sp.. However, in 1979 the true species diversity of *Boophis* was not fully understood (Glaw et al. 2001) and matching of tadpoles to species was difficult without molecular genetic techniques, which resulted in equivocal identity of the tadpoles from these early studies. Tadpoles assigned by Blommers-Schlösser (1979) to *Boophis majori* probably belong to *B. marojezensis*, and the identity of tadpoles assigned to *B. erythrodactylus* remains uncertain, because this species belongs to a species group which has generalized tadpoles. Subsequent to these early works, Raharivololoniaina et al. (2006) described the tadpoles of *B. marojezensis* and *B. sibilans* from Andasibe. Glos et al. (2007) described *B. schuboeae* tadpoles from Ranomafana and *B. ankaratra* tadpoles from Andringitra, and Thomas et al. (2006) described *B. andohahela* tadpoles from Ranomafana. More recently, Rasolonjatovo et al. (2010) described the larvae of *B. englaenderi*, *B. luciae*, and *B. vittatus*.

In this study, morphological data on twenty-two strongly rheophilous tadpoles are provided, of which fourteen were previously unknown. Twelve of these larvae belong to candidate species which so far have not been scientifically named.

All these strongly rheophilous tadpoles are characterized by their “streamlined” (i.e., elongated, narrow and flat) body form, their wide oral disc containing many keratodont rows with all posterior rows uninterrupted, their completely keratinized jaw sheaths, of which the lower one is always “ribbed” and the upper one can be absent in some species, and rows of many small rounded marginal papillae with or without a dorsal gap. The absence of many of these characteristics in *B. williamsi* tadpoles (Blommers-Schlösser 1979 and Schmidt et al. 2008) is the criteria of excluding them from the present study.

In the context of grouping Malagasy tadpoles into different ecomorphological guilds, some *Boophis* tadpoles have been classified as “suctorial” and “adherent” by Altig and Johnston (1989), and Raharivololoniaina et al. (2006) have classified other *Boophis* tadpoles more in detail according to their morphological characters. However, for a definition of ecomorphological guilds, it is appropriate to include also ecological data. Therefore, we here combine our morphological descriptions with the results of an ecological analysis of the three most abundant strongly rheophilous tadpoles in Ranomafana National Park in the southern central east of Madagascar (*B. luciae*, *B. marojezensis* [Ca51], and *B. andohahela*). Based on habitat characteristics from 30 streams in this rainforest reserve we tested whether the typical morphological characteristics of these tadpoles are indeed associated with a preference for a fast-running stream habitat, as it has been predicted by Blommers-Schlösser (1979).

The use of DNA barcoding to identify amphibian larvae from species-rich tropical communities to the species level has been tremendously successful within the last years (e.g., Thomas et al. 2005). It also has induced a so called “reverse taxonomy” in this vertebrate group (Randrianiana et al. 2011a), with species new to science discovered first by their larvae rather than their adult stage. The present study confirms this progress by discovering twelve candidate species via tadpole DNA sequences and morphology, many of which are still unknown in their adult stage.

## Materials and methods

### Morphological study of tadpoles

Tadpoles were collected by different kinds of nets having mesh sizes from 2 to 5 mm, depending on the size of the streams, the strength of the water current and the type of substrate. They were euthanised by immersion in chlorobutanol solution, and immediately sorted into homogeneous series based on morphological characters (body shape, relative tail length, eye position and direction, oral disc position, direction and configuration, general color pattern). From each series one specimen was selected and a tissue sample from its tail musculature or fin taken and preserved in 99% ethanol. This specimen is here called “DNA voucher”. All detailed morphological tadpole characterizations and drawings are based on this DNA voucher, whereas variation is sometimes described based on further specimens of the series. After tissue collection, all specimens were preserved in 5% formalin or 70% ethanol. Specimens were deposi-

ted in the Zoologische Staatssammlung München, Germany (ZSM). When referring to voucher specimens the original field numbers (FG/MV, FGZC, T, and ZCMV) are usually provided together with the final ZSM catalogue numbers. Tadpoles studied in this paper are summarized in Tables 1 and 2, including data concerning the site and its coordinates, the date of the capture and the collectors.

For detailed morphological examination, especially to determine developmental stages and assess characters of the oral disc, preserved tadpoles were stained slightly with methylene blue. Tadpoles were examined under water, and a few drops of methylene blue were applied to the oral disc, hind limb, spiracle, narial opening and vent tube to better discern their structures. Developmental stages were determined following Gosner (1960).

Morphological descriptions, measurements and drawings were done on digital pictures of the preserved tadpoles taken with a stereomicroscope Zeiss StereoDiscovery V12 connected to a computer, following landmarks, terminology and definitions of Altig and McDiarmid (1999) and Randrianaina et al. (2011a), except that we predominantly use the term keratodonts instead of labial teeth. The formula of keratodont rows (labial tooth row formula, LTRF) is given according to Altig and McDiarmid (1999). Comparing measurements, we consider them as “almost equal” if ratios of the measured values are 95–96% or 104–105%, “equal” if they are in the range 97–103%, as “almost in the middle” if they are in the range 45–46% or 54–55% and “in the middle” if they are in the range 47–53% (Randrianaina et al. 2011a). All the measurement data are summarized in Tables 3 to 5 electronic supplement. Most of these data, especially concerning the oral disc, are used for elaborating morphological clusters, into which all tadpoles are classified. The following abbreviations are used:  $A_1$  (first upper keratodont row),  $A_2$  (second upper keratodont row),  $A_{2\text{gap}}$  (medial gap in  $A_2$ ),  $A_3$  (third upper keratodont row),  $A_4$  (fourth upper keratodont row),  $A_5$  (fifth upper keratodont row),  $A_6$  (sixth upper keratodont row),  $A_7$  (seventh upper keratodont row),  $A_8$  (eighth upper keratodont row),  $A_{1-8\text{ den}}$  (density of the keratodonts in row  $A_{1-8}$ ),  $A_{1-8\text{ len}}$  (length of  $A_{1-8}$ ),  $A_{1-8\text{ num}}$  (number of keratodonts in  $A_{1-8}$ ), BH (maximal body height), BL (body length), BW (maximal body width), DF (dorsal fin height at midtail), DG (size in rows of the dorsal gap of marginal papillae), DMTH (distance of maximal tail height from the tail-body junction), ED (eye diameter), EH (eyes height – measured from the lower curve of the belly), HAB (height of the point where the axis of the tail myotomes contacts the body – measured from the lower curve of the belly), IND (inter-narial distance), IOD (inter-orbital distance), JW (maximal jaw sheath width), MC (medial convexity of the upper sheath), MCL (length of the medial convexity of the upper sheath), MP (marginal papillae), MTH (maximal tail height), ND (naris diameter), NH (naris height - measured from the lower curve of the belly), NP (naris-pupil distance), OD (oral disc), ODW (maximum oral disc width),  $P_1$  (first lower keratodont row),  $P_2$  (second lower keratodont row),  $P_3$  (third lower keratodont row),  $P_{1-3\text{ den}}$  (density of the keratodonts in  $P_{1-3}$ ),  $P_{1-3\text{ len}}$  (length of  $P_{1-3}$ ),  $P_{1-3\text{ num}}$  (number of keratodonts in  $P_{1-3}$ ), PCA (Principal Component Analysis), RN (rostro-narial distance), SBH (distance between snout and the point of maximal body height), SBW (distance between snout and the point of maximal body width), SE (snout-eye distance), SH (spiracle height – measured from the lower curve of the belly), SL (spiracle length), SMP (submarginal



**Table 1.** Summary of localities with geographic coordinates, and collection dates, of tadpole specimens studied herein.

Locality	Site	Species	Coordinates	Date	Collectors
Ankijagna Lalagna		<i>B. sambirano</i> [Ca49]	14°14.055'S 48°58.732'E 1187 m a.s.l.	08.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiana
Ambohitsara		<i>B. albipunctatus</i>	21°21.431'S 47°48.941'E 294 m a.s.l.	03.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiana
Ambinanitelo		<i>B. marojezensis</i> [Ca52]	14°13.524'S 48°57.808'E 1182 m a.s.l.	09.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiana
Ambinanitelo		<i>B. sambirano</i> [Ca50]	14°13.524'S 48°57.808'E 1182 m a.s.l.	09.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiana
An'Ala	Andohanisity	<i>B. mandraka</i> [Ca46]	18°55.156'S 48°29.278'E 889 m a.s.l.	08.02.2006	C. Patton, D.R. Vieites, J. Patton, L. Raharivololoniaina, M. Vences, R.D. Randrianiana
Andasibe Special Reserve	Analamazaotra river	<i>B. sibilans</i>	18°55.900'S 48°25.733'E 900 m a.s.l.	04.12.2001	L. Raharivololoniaina, M Vences
Between Antsohihy and Bealanana	Anjingo river	<i>B. sambirano</i> [Ca47]	14°44.929'S 48° 29.491'E 925 m a.s.l.	07.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiana
Between Antsohihy and Bealanana	Anjingo river	<i>B. sambirano</i> [Ca48]	14°44.929'S 48°29.491'E 925 m a.s.l.	07.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A . Rakotoarisoa, M. Vences, R.D. Randrianiana
Manongarivo Special Reserve	Camp Norbert	<i>B. sambirano</i>	13°56.053'S 48°27.028'E 288 m a.s.l.	31.01.2003	F. Glaw, M. Vences, R.D. Randrianiana
Marojejy National Park	Camp Mantella	<i>B. vittatus</i>	14°26.972'S 49°47.214'E 327 m a.s.l.	14.02.2005	F. Glaw, M. Vences, R.D. Randrianiana
Marojejy National Park	Camp Marojejia	<i>B. englaenderi</i>	14°26.070'S 49°45.638'E 740 m a.s.l.	18.02.2005	F. Glaw, M. Vences, R.D. Randrianiana

Locality	Site	Species	Coordinates	Date	Collectors
Marojejy National Park	Camp Mantella	<i>B. englaenderi</i> [Ca23]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiana
Marojejy National Park	Camp Mantella	<i>B. marojezensis</i> [Ca25]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiana
Marojejy National Park	Camp Mantella	<i>B. marojezensis</i> [Ca26]	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiana
Marojejy National Park	Camp Mantella	<i>B. sibilans</i>	14°26.972'S 49°47.214'E 327 m a.s.l.	19.02.2005	F. Glaw, M. Vences, R.D. Randrianiana
Ranomafana National Park	Ambatolahy river	<i>B. andohahela</i>	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiana
Ranomafana National Park	Ambatolahy river	<i>B. marojezensis</i> [Ca51]	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiana
Ranomafana National Park	Ambatolahy river	<i>B. schuboeae</i>	21°14.897'S 47°25.769'E 867 m a.s.l.	27.07.2009	R.D. Randrianiana
Ranomafana National Park	Imaloka	<i>B. marojezensis</i> [Ca51]	21°14.529'S 47°27.938'E 957 m a.s.l.	01.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiana
Ranomafana National Park	In a pool below waterfall	<i>B. schuboeae</i>		11.02.2003	M. Teschke, M. Vences
Ranomafana National Park	Marihy avaratra	<i>B. luciae</i>	21°15.806'S 47°25.548'E 1144 m a.s.l.	20.02.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiana
Ranomafana National Park	Marihy avaratra	<i>B. mandraka</i> [Ca38]	21°15.806'S 47°25.548'E 1144 m a.s.l.	02.02.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianiana
Ranomafana National Park	Talatakely	<i>B. luciae</i>	21°15.846'S 47°25.161'E 966 m a.s.l.	24.02.2006	L. Raharivololoniaina, A.F. Ranjanaharisoa, T.J. Razafindrabe, D.R. Vieites, J. Patton, C. Patton, M. Vences, R.D. Randrianiana
Ranomafana National Park	Talatakely	<i>B. marojezensis</i> [Ca51]	21°15.846'S 47°25.161'E 966 m a.s.l.	24.02.2006	L. Raharivololoniaina, A.F. Ranjanaharisoa, T.J. Razafindrabe, D.R. Vieites, J. Patton, C. Patton, M. Vences, R.D. Randrianiana

Locality	Site	Species	Coordinates	Date	Collectors
Ranomafana National Park	Sahateza (Pond Donald)	<i>B. ankaratra</i>	21°15.476'S 47°21.583'E 1016 m a.s.l.	03.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianaina
Ranomafana National Park	Vatoharana	<i>B. andohahela</i>	21°17.338'S 47°25.765'E 1016 m a.s.l.	24.03.2007	A. Strauß, J. Glos, E. Reeve, T. Rasolonjatovo-H., S. Ndriantsoa, M. Vences, R.D. Randrianaina
Tsaratanana Strict Nature Reserve	Antevialambazaha	<i>B. marojezensis</i> [Ca53]	14°10.455'S 48°56.714'E 1699 m a.s.l.	10.06.2010	D.R. Vieites, F.M. Ratsoavina, A.S. Rasamison, A. Rakotoarisoa, M. Vences, R.D. Randrianaina

**Table 2.** Collection numbers and Genbank accession numbers of the tadpoles studied. FG/MV, FGZC, LR, T, TAD, ZCMV (field numbers), ZSM (Zoologische Staatssammlung München). Missing accession numbers indicate that sequences were too short or of poor quality and were therefore not submitted to Genbank, or that they will be submitted to Genbank in the course of future studies.

Species	Locality	ZSM- and Field number	Accession number
<i>Boophis englaenderi</i>	Marojejy National Park	FGZC 2244- ZSM 623/2008	HM769921
<i>B. englaenderi</i> [Ca23]	Marojejy National Park	FGZC 2957- ZSM 1632/2007	JQ518193
		FGZC 2241- ZSM 1499/2007	---
		FGZC 2243- ZSM 527/2008	FJ559144
		FGZC 2248- ZSM 1508/2007	---
		FGZC 2250- ZSM 1502/2007	---
		FGZC 2252- ZSM 1503/2007	---
		FGZC 2257- ZSM 529/2008	---
		FGZC 2260- ZSM 530/2008	---
		FGZC 2273- ZSM 1514/2007	---
		FGZC 2275- ZSM 1516/2007	---
<i>Boophis andohahela</i>	Ranomafana National Park	T 60- ZSM 912/2007	GU974437
		T 107- ZSM 1319/2007	GU974422
		T 125- ZSM 1321/2007	GU974423
		T 127- ZSM 1162/2007	GU974424
		T 131- ZSM 1351/2007	GU974425
		T 150- ZSM 910/2007	GU974427
		T 222- ZSM 566/2007	GU974435
		T 428- ZSM 998/2007	GU974449
		T 09/273- ZSM 282/2009	---
<i>Boophis ankaratra</i>	Ranomafana National Park	FGMV 2003.1698- ZSM 816/2004	---
		ZCMV 3803- ZSM 168/2008	---
		ZCMV 4917- ZSM 876/2007	GU974476

Species	Locality	ZSM- and Field number	Accession number
<i>Boophis schuboeae</i>	Ranomafana National Park	FGMV 2002.1800- ZSM 978/2004	DQ068394
		Tad 2004-780- ZSM 1339/2004	---
		Tad 2004-797- ZSM 1356-2004	---
		T 09/980- ZSM 743/2008	---
		T 09/968- ZSM 739/2008	---
		T 09/971- ZSM 740/2008	---
		T 09/998- ZSM 749/2008	---
<i>B. albipunctatus</i>	Ambohitsara	ZCMV 4942- ZSM 78/2008	GU974373
		ZCMV 4946- ZSM 82/2008	GU974374
<i>Boophis sibilans</i>	An'Ala	ZCMV 3450- ZSM 1754/2007	---
<i>Boophis sibilans</i>	Andasibe	LR 269- ZSM 557/2004	DQ792492
<i>Boophis sibilans</i>	Marojejy National Park	FGZC 2956- ZSM 1631/2007	JQ518194
<i>Boophis luciae</i>	Ranomafana National Park	T 176- ZSM 792/2007	---
		T 177- ZSM 593/2007	GU975090
		T 178- ZSM 541/2007	GU975094
		T 179- ZSM 976/2007	---
		T 224- ZSM 264/2007	---
		T 430- ZSM 274/2007	GU975096
		ZCMV 3619- ZSM 1587/2006	HM769939
		ZCMV 3631- ZSM 1588/2006	HM769940
		ZCMV 3686- ZSM 634/2008	HM769938
		ZCMV 4024- ZSM 688/2007	---
		ZCMV 5146- ZSM 730/2007	---
<i>B. sambirano</i>	Manongarivo Special Reserve	FGMV 2002.1904- ZSM 678/2004	EU717863
		FGMV 2002.1902- ZSM 672/2004	EU717861
<i>B. mandraka</i> [Ca38]	Ranomafana National Park	ZCMV 4261- ZSM 456/2007	FJ559153
<i>B. mandraka</i> [Ca46]	An'Ala	ZCMV 3479- ZSM 1784/2007	JQ518195
<i>B. sambirano</i> [Ca47]	Between Antsohihy and Bealanana	ZCMV 13105- ZSM 482/2010	JQ518203
		ZCMV 13110- ZSM 486/2010	JQ518204
<i>B. sambirano</i> [Ca48]	Between Antsohihy and Bealanana	ZCMV 13107- ZSM 484/2010	JQ518206
		ZCMV 13108- ZSM 485/2010	JQ518207
		ZCMV 13109- ZSM 485/2010	JQ518205
<i>B. sambirano</i> [Ca49]	Ankijagna Lalagna	ZCMV 13150- ZSM 523/2010	JQ518209
		ZCMV 13155- ZSM 528/2010	JQ518208
		ZCMV 13156- ZSM 529/2010	JQ518210
<i>B. sambirano</i> [Ca50]	Ambinanitelo	ZCMV 13171- ZSM 544/2010	JQ518212
		ZCMV 13172- ZSM 545/2010	JQ518211
		ZCMV 13173- ZSM 546/2010	JQ518213
		ZCMV 13174- ZSM 547/2010	JQ518214
<i>B. marojezensis</i>	Marojejy National Park	FGZC 2277- ZSM 1528/2007	JQ518196
		FGZC 2953- ZSM 1628/2007	JQ518199
<i>B. marojezensis</i> [Ca25]	Marojejy National Park	FGZC 2929- ZSM 1611/2007	FJ559146
<i>B. marojezensis</i> [Ca26]	Marojejy National Park	FGZC 2930- ZSM 1612/2007	JQ518197
<i>B. marojezensis</i> [Ca51]	Ranomafana National Park	T 394- ZSM 1008/2007	GU974657
		T 432- ZSM 117/2007	GU974658
		T 09/1088- ZSM 779/2008	---



Species	Locality	ZSM- and Field number	Accession number
		T 09/1091- ZSM 780/2008	---
		T 09/1094- ZSM 781/2008	---
		ZCMV 3691- ZSM 267/2008	---
		ZCMV 3629- ZSM 318/2008	---
		ZCMV 3635- ZSM 232/2008	---
		ZCMV 3690- ZSM 266/2008	---
		ZCMV 3742- ZSM 481/2008	---
		ZCMV 4203- ZSM 401/2007	---
		ZCMV 4264- ZSM 457/2007	GU974654
		ZCMV 4376- ZSM 1453/2007	GU974647
		ZCMV 4531- ZSM 532/2007	GU974648
		ZCMV 4541- ZSM 504/2007	GU974650
		ZCMV 4547- ZSM 1390/2007	GU974651
		ZCMV 4550- ZSM 509/2007	GU974652
		ZCMV 4931- ZSM 838/2007	GU974656
		ZCMV 5098- ZSM 913/2007	GU974646
		ZCMV 5986- ZSM 1212/2007	GU974655
		ZCMV 1395- ZSM 0025/2007	GU974653
		T 09/1085- ZSM 778/2008	---
<i>B. marojezensis</i> [Ca52]	Ambinanitelo	ZCMV 13168- ZSM 541/2010	JQ518215
		ZCMV 13169- ZSM 542/2010	---
<i>B. marojezensis</i> [Ca53]	Tsaratanana Strict Nature Reserve	ZCMV 13200- ZSM 573/2010	JQ518216
		ZCMV 13201- ZSM 574/2010	---
		ZCMV 13202- ZSM 575/2010	---
		ZCMV 13203- ZSM 576/2010	---
		ZCMV 13204- ZSM 577/2010	---
		ZCMV 13205- ZSM 578/2010	JQ518217
<i>Boophis vittatus</i>	Marojejy National Park	FGZC 2237- ZSM 5219/2005	---
		FGZC 2238- ZSM 1906/2007	JQ518200
		FGZC 2251- ZSM 1907/2007	JQ518201
		FGZC 2914- ZSM 1601/2007	JQ518202

papillae), SS (snout-spiracle distance), SV (spiracle-vent distance), TAL (tail length), TH (tail height at the beginning of the tail), THM (tail height at midtail), Thorn-pap (thorn-shaped papillae), TL (total length), TMH (tail muscle height at the beginning of the tail), TMHM (tail muscle height at midtail), TMW (tail muscle width at the beginning of the tail), LR (number of the lower rows of keratodonts), UR (number of the upper rows of keratodonts), VF (ventral fin height at midtail), VG (size in rows of the ventral gap of marginal papillae), VL (vent tube length).

In tadpoles of many frog species, pigmentless parts of the body wall become detached and apparently separated by a liquid-filled cavity from the underlying pigmented parts of the skin and the inner organs (among Malagasy frogs, for instance extremely expressed in the tadpoles of some *Scaphiophryne*; see Grosjean et al. 2007). These cavities probably represent lymphatic sacs or sinuses but this hypothesis has not

been verified in most anuran species. In the rheophilous *Boophis* tadpoles, the extension of this detachment of a transparent part of the body wall appears to be characteristic for some species and candidate species. The difference often refers to the extension and ease to recognize this transparent area of the body wall, and we therefore use terms like recognizable vs. poorly recognizable rather than present vs. absent when referring to this structure, although there are clearly pronounced differences in its expression among some species.

### **DNA-based species identification**

DNA barcoding was based on a fragment of the mitochondrial 16S rRNA gene, which is known to be sufficiently variable among species of Malagasy frogs (Vences et al. 2005). We amplified a fragment of ca. 550 bp using primers 16Sar-L and 16Sbr-H from Palumbi et al. (1991), or a shorter fragment of ca. 400 bp using the newly developed specific mantellid primers 16S-Frog-L1 (CAT AAT CAC TTG TTC TTT AAA) and 16S-Frog-H1 (GAT CCA ACA TCG AGG TCG). PCR was carried out with standard protocols (Vences et al. 2005) and sequences resolved on automated sequencers. Sequences were preliminarily identified using BLAST searches against a near-complete database of sequences of adult Malagasy frog species. Results were subsequently verified by manually aligning and comparing sequences to the closest hits in the database. Identification was considered to be unequivocal when the tadpole sequence was 99–100% identical to an adult specimen from the same geographical region, and clearly less similar to all sequences from other species. When no identity with adult specimens was found and divergence was >3% we considered the corresponding tadpoles to belong to undescribed candidate species. Newly determined DNA sequences were deposited in Genbank (accession numbers JQ518193- JQ518217).

Candidate species nomenclature followed the scheme developed by Padial et al. (2010). We use the binomial species name of the closely related species, followed in square by the abbreviation “Ca” with an attached numerical code referring to the particular candidate species, and at first mention terminating with the author name and the year of publication of the article in which the lineage was first discovered for few species, or the Genbank accession number of a DNA sequence of a reference specimen for others. Further in the text, we abbreviate the candidate species name just by using the binomial species name followed in square brackets by the abbreviation “Ca” and its numerical code.

### **Ecological study of tadpoles**

During a study on stream tadpole communities in Ranomafana National Park (RNP) in the south eastern escarpment of Madagascar, we exhaustively sampled 33 stream sections for tadpoles (Strauß et al. 2010). Each section spanned 30 m and the sampling

process was conducted separately for all available microhabitats within the section. We aimed to exhaustively sample tadpoles using dip nets of different sizes and materials, adjusted to obtain optimal sampling results for each stream. Sampling started downstream, and depending on stream width two to five people processed slowly on the same level upstream while dip-netting as much as reasonably possible all tadpoles in all microhabitats. These microhabitats were predefined subject to underground substrate (rock, gravel, leaves, sand) as well as separately by the stream velocity categories “fast” (obviously running) and “slow” (almost stagnant). Habitat variables were recorded at two spatial levels: (1) habitat variables of possible importance for breeding site (stream) choice of frog species and (2) proportion of microhabitats available within the streams.

We used data from this study for an exemplary analysis of breeding site choice and microhabitat use of syntopic species of strongly rheophilous tadpoles. To identify the habitat variables of the stream and the surrounding forest that may be important for breeding site choice, we performed a principal component analysis (PCA) and plotted species according to their incidence as supplementary variables in the PCA biplot. For PCA, we used all ten habitat variables of all 33 streams sampled during the tadpole community study. PCA was run on the correlation matrix in order to standardize for the influence of unequal variance. To evaluate data outliers and linear interdependence of variables, box-plots and pair-plots (Zuur et al. 2007) were used. As PCA requires multinormality of data, box-cox-power-transformations (Box and Cox 1964) were applied when necessary. The number of meaningful PCs was estimated by a scree plot (Zuur et al. 2007). PCA and correlation with species incidence was evaluated using the *dimdesc* function in package *FactoMineR* (Lê et al. 2008).

To analyze the use of microhabitats within streams, we first constructed graphs of raw data to display the species specific distribution between microhabitats. In order to quantify true preferences for microhabitats, Ivlev's electivity index ( $E$ , Ivlev 1961) was calculated for each strongly rheophilous *Boophis* species occurring in RNP.  $E$  is defined as  $E = (r - p) / (r + p)$  with  $r$  being the proportions of the microhabitats used and  $p$  the proportion of microhabitats available. To test whether the  $E$  values differ for the single species, a factorial ANOVA was run with  $E$  as dependent variable and the factors “microhabitat” and “species” as independent variables. This provides information whether  $E$  is different for the different microhabitats, whether  $E$  differs between species, and, if interactions could be included in model, whether the effect of the one factor depends on the level of the other factor. To avoid possible overparameterisation caused by large numbers of interactions (Crawley 2007), we removed the interaction term from the model and performed ANOVAs of subsets of the data to closer evaluate differences in preferences between species within specific microhabitats (interactions). Only the three abundant species were included in this analysis. Also, for each species only streams with at least eight specimens of the respective species were included in the analysis to reduce the influence of many high avoidance values due to a general low number of tadpoles in a stream.

Statistical analysis were performed in R 2.9.2 (R Development Core Team 2009) including libraries *car* (Fox et al. 2008) and *FactoMineR* (Lê et al. 2008).

## Results

### Tadpole descriptions

We here provide a summary of the most important morphological characteristics of one representative species per species group, and brief accounts for all other species and candidate species in which we mainly emphasize their difference to the species described more completely, or to other species belonging to the same group. Standardized, detailed descriptions and assessments of variation for all species and candidate species are found in the electronic supplement. Original measurements and ratios are given in Tables 3–5 which are equally included as electronic supplement.

### *Boophis luteus* group

This group is characterized by tadpoles having a generalized oral disc without lateral emargination and ventral gap of papillae, but the dorsal gap is wide to very wide. The anterior margin of the oral disc is a continuation of the snout. Usually  $A_1$  is uninterrupted and  $P_1$  is interrupted, except the three species described herein which are the ones in the group having the strongest expression of adaptations that we interpret as rheophilous. The jaw sheaths are very strong with smooth surface and completely or partially keratinized in some species. The upper sheath is always made up by a medial convexity. Dorsolateral glands which exist in some other *Boophis* tadpoles are absent.

### *Boophis englaenderi* Glaw & Vences, 1994

Morphological data were assessed in one tadpole (Figures 2 and 3) in developmental stage 36 (field number FGZC 2244; ZSM 623/2008, BL 11.8 mm, TL 25.4 mm, accession number HM769921) from Marojejy National Park (previously described by Rasolonjatovo Hiobiarilanto et al. 2010). The 16S rDNA sequence of this specimen is 99.5% identical to a reference sequence of an adult *B. englaenderi* (accession FJ559124) from Marojejy.

The tadpoles of this species have an elliptical body, a flatly rounded snout in dorsal view and a short tail. The distance between eyes is wide and nares are very large, round, positioned very high dorsally, and situated nearer to snout than to eye and at eye level. LTRF is 6(3–6)/3(1). The upper jaw sheath is totally keratinized with rounded serrations, moderately wide with a very short widely rounded medial convexity. The lower sheath is V-shaped, completely keratinized and partially hidden by the upper one. Both jaw sheaths have a smooth surface.

In preservative, the tadpole is generally dark brown. Dark brown spots condensed to form a hexagonal mark above the neurocranium; a dark semicircular patch situated posterior to each narial opening and dark patches between the vertebral area and the

abdominal region are present. The snout is spotted. The transversal lines between the vertebral area and the abdominal region are perceivable which make the domino-like structure on this noticeable. The dorsal part of the tail muscle has five dark brown and four light alternating bands. The prominent dark brown band is the extension of the patches between the vertebral area and the abdominal region. The myosepta are visible on the dorsal part of the tail. Laterally, the jugal area is covered by dense dark brown patches and the dorsolateral part of the flank is identical to the dorsal pattern; the ventrolateral part is pale and the abdominal region is very dark leaving an opaque discernible spiracle. Ventrally, oral disc, gular and branchial regions are pale; the venter is more or less transparent and the intestinal coils are perceptible with a regularly spiral shape. The tail musculature is pale and covered by dark brown spots which condense to form reticulations. Fins are transparent, with few brown spots on the dorsal fin, and the ventral fin is free from pigment.

### ***Boophis englaenderi* [Ca23 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 4) in developmental stage 30 (field number FGZC 2957, ZSM 1632/2007, BL 10.5 mm, TL 29.5 mm, Genbank accession number JQ518193) from Marojejy National Park. The 16S rDNA sequence of this specimen is 94% identical to a reference sequence of an adult *B. englaenderi* (accession AY848474) from Ilampy. Nine other voucher specimens agree in morphology with the voucher specimen described herein.

The external morphology of this tadpole has a very close similarity with that of *B. englaenderi*, except that it has a distinctly longer tail (TAL/BL 183% *vs.* 153%) and a lighter pigmentation. Additional differences between the two tadpoles are found in the oral disc structure. It is bulged laterally and has one more interrupted upper keratodont row and a first uninterrupted lower row giving the keratodont row formula LTRF 7(3–7)/3 *vs.* 6(3–6)/3(1). The number of papillae is higher than in *B. englaenderi* with 175 marginal papillae (*vs.* 128), and 94 submarginal papillae (*vs.* 33), although the examined tadpole is still in a developmental stage inferior to that of the examined tadpole of *B. englaenderi*. The submarginal papillae are complete on the lower labium. This tadpole is also characterized by a light brown coloration in preservative. The jugal area is covered by scarce light brown patches, and the tail musculature is covered by light brown spots which group in some areas to form patches or sparse reticulations. The intestinal coils are visible. The examination of nine other voucher specimens (see Table 2) confirms the differences to *B. englaenderi*.

### ***Boophis andohahela* Andreone, Nincheri & Piazza, 1995**

Morphological data were assessed in one tadpole (Figures 2 and 5) in developmental stage 26 (field number T 428; ZSM 998/2007, BL 11.8 mm, TL 25.4 mm, Genbank



accession number GU974449) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. andohahela* (accession AY848456) from the same locality. Five out of six other voucher specimens have the morphological characteristics of this species, whereas one tadpole has a difference in the oral disc configuration.

The general morphology of this tadpole is similar to that of *B. englaenderi* and *B. englaenderi* [Ca23], but it is characterized by the presence of a white patch posterior to the hexagonal mark above the neurocranium in life and even in preservative (Figure 1). The non-visibility of its intestinal coils is shared with *B. englaenderi*. The LTRF 6(3–6)/3 is identical to that of some specimens of *B. englaenderi* but differs from that of *B. englaenderi* [Ca 23]. On the other hand, the absence of papillae on the ventral area of the lower labium is similar to that of *B. englaenderi*. The oral disc of this tadpole has a slightly developed lateral bulge.

### ***Boophis albipunctatus* group**

This group is characterized by tadpoles having an enlarged oral disc without lateral emargination (but bulged laterally in some species) and ventral gap of marginal papillae. The dorsal gap is moderately wide. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the entire margin is free from the snout. LTRF 8(5–8)/3 or 7(5–7)/3. The jaw sheaths are moderately strong and completely keratinized. The upper sheath has a medial convexity in some species. The lower sheath is U or V-shaped and ribbed. Dorsolateral glands are present.

### ***Boophis ankaratra* Andreone, 1993**

Morphological data were assessed in one tadpole (Figures 2 and 6) in developmental stage 28 (field number ZCMV 4917, ZSM 876/2007, BL 11.3 mm, TL 25.5 mm, Genbank accession number GU974476) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. ankaratra* (accession AJ315909) from Mandraka. Two other voucher specimens possess the typical morphological characters of the species.

This tadpole can be differentiated from *B. luteus* group tadpoles by the general state of the oral disc. It is characterized by an enlarged and laterally bulged oral disc. There is a double row of marginal papillae interrupted by a moderately wide dorsal gap. Papillae are short, small, conical with protuberance, and their tip is rounded. There are 148 and 190 marginal and submarginal papillae, respectively. The LTRF is 8(5–8)/3 and  $A_1$  is moderately long. The jaw sheaths are moderately strong and totally keratinized. The upper sheath is characterized by a short narrowly pointed medial convexity. The lower sheath is U-shaped, ribbed, higher than wide, and partially hidden by the upper one.

In life this tadpole is generally dark brown. Dorsally, body and tail covered by dense brown spots. A hexagonal mark above the neurocranium and a dark semicircular patch posterior to each narial opening are obvious. The domino-like structures between the vertebral area and the abdominal region are recognizable. Few irregular dark blotches and silvery spots scattered on the skin. Laterally, jugal area is covered by dense brown patches and the abdominal region is very dark leaving a transparent noticeable spiracle. The tail musculature is yellowish and covered by sparse brown spots which coalesce to form patches. Their density diminishes toward the tail tip. Fins are transparent with few brown blotches on the dorsal fin and the ventral fin is almost free from pigment. Ventrally, intestinal coils are invisible (Figure 1). In preservative, the tadpole is similar except that it is paler and the silver tissue which covers the heart and the venter has become whitish.

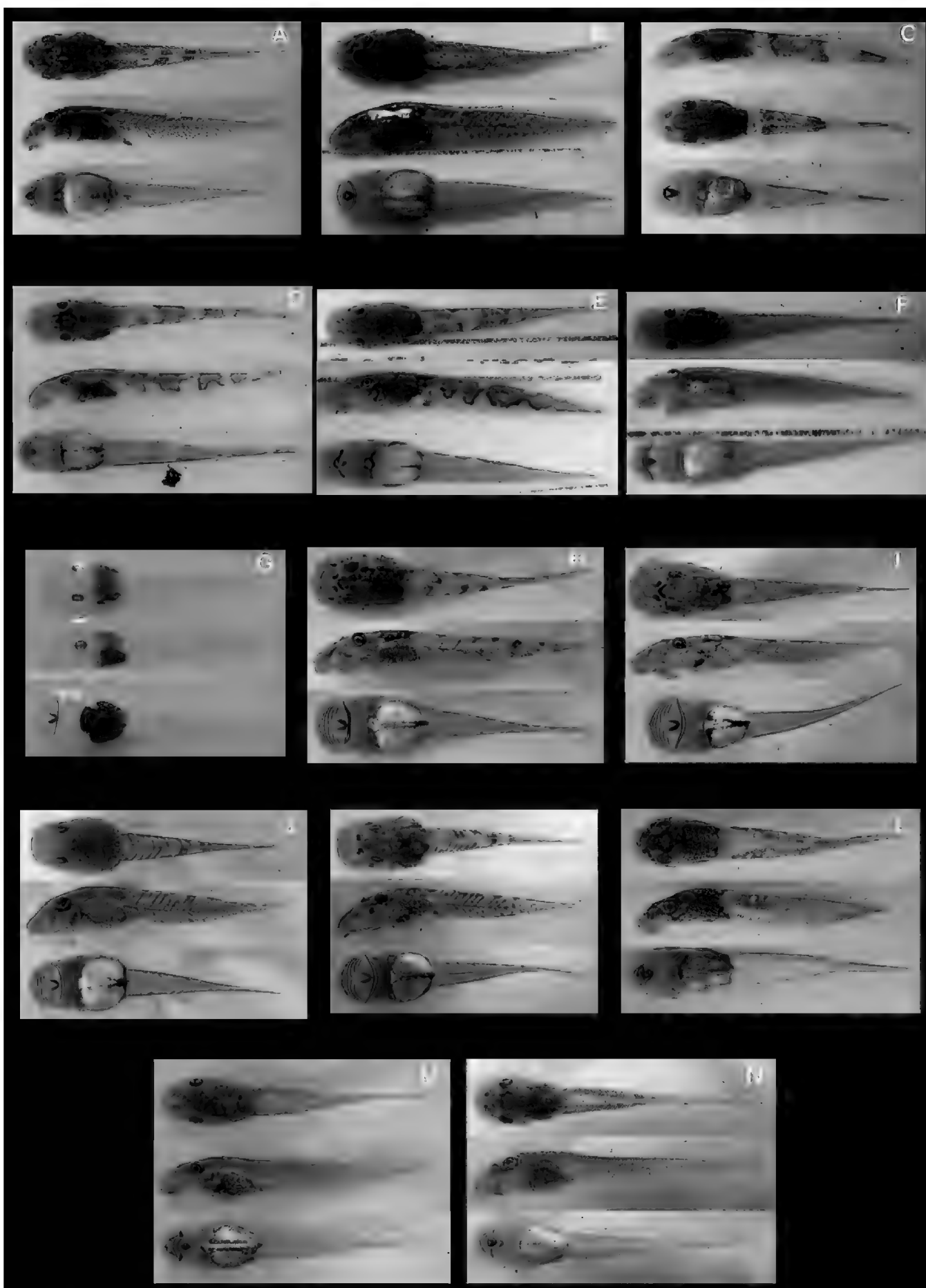
### ***Boophis schuboeae* Glaw & Vences, 2002**

Morphological data were assessed in one tadpole (Figures 2 and 7) in developmental stage 36 (field number FGMV 2002–1800, ZSM 978/2004, BL 12.1 mm, TL 25.5 mm, Genbank accession number DQ068394) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult *B. schuboeae* (accession AJ315912) from the same locality. Six other voucher specimens from the same locality show the typical coloration pattern and oral disc configuration of the species.

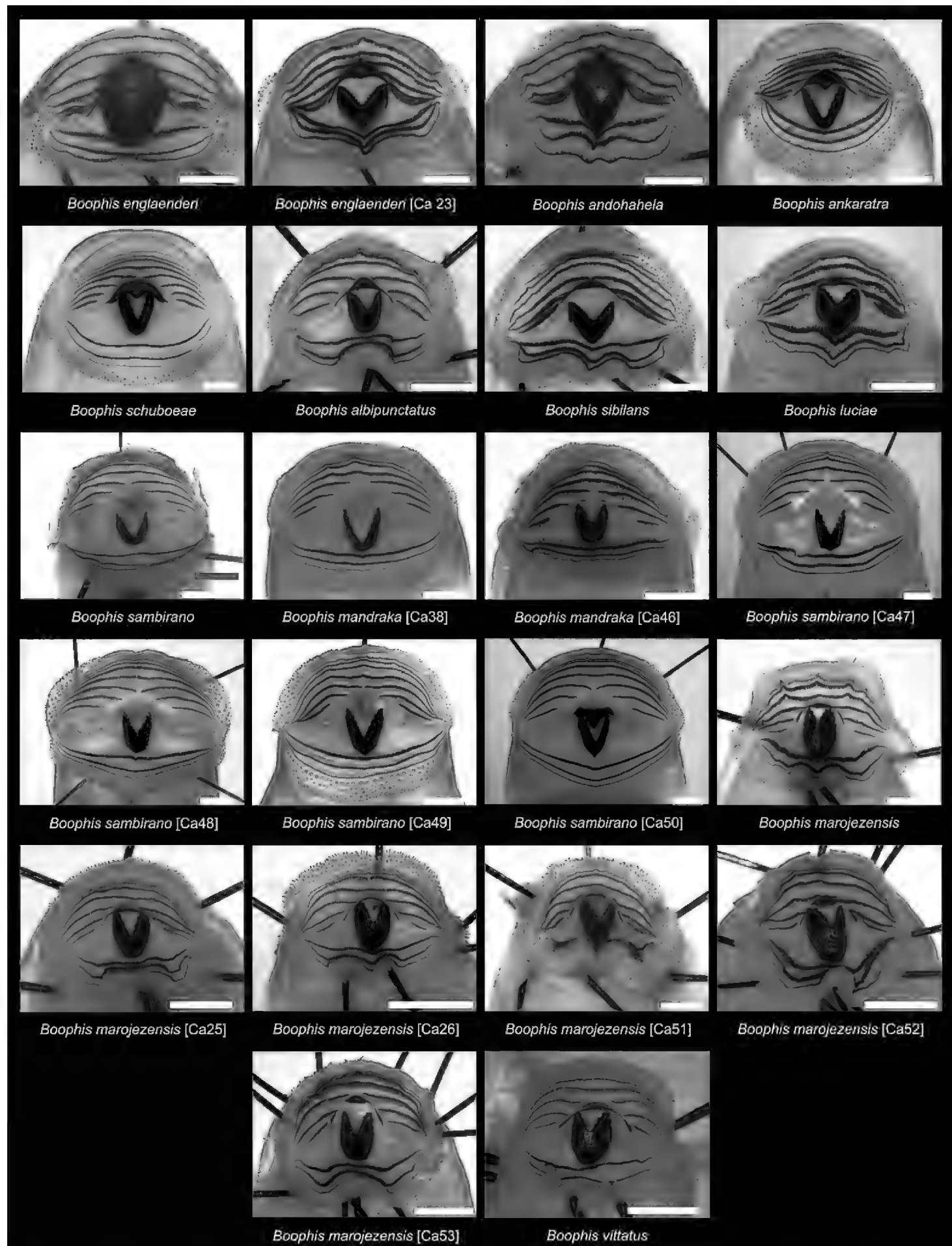
The oral disc of the tadpoles belonging to this species is identical to those of *B. ankaratra*, except that it has a lower number of rather smaller papillae, and the lateral area where the oral disc folds is free from submarginal papillae. However, the tadpoles of these two species are easy to distinguish by their particular coloration pattern (Figure 1) (see also Glos et al. 2007). *B. schuboeae* tadpoles are characterized by the presence of up to four light and three alternating dark bands on the tail musculature. In life, the posterior part of the tail is sometimes with a contrasting orange coloration. Typically the dorsal and ventral fins originate on the tail musculature for *B. schuboeae* while they commonly originate on the body-tail junction for *B. ankaratra*.

### ***Boophis albipunctatus* Glaw & Thiesmeier, 1993**

Morphological data were assessed in one tadpole (Figures 2 and 8) in developmental stage 25 (field number ZCMV 4946, ZSM 82/2008, BL 7.5 mm, TL 15.5 mm, Genbank accession number GU974374) from Ambohitsara-Tsitolaka. The 16S rDNA sequence of this specimen is 99% identical to a reference sequence of a *B. albipunctatus* adult specimen (accession AY848446) from Manantantely. One other voucher tadpole of *B. albipunctatus* from the same locality is morphologically very similar to the described voucher specimen.

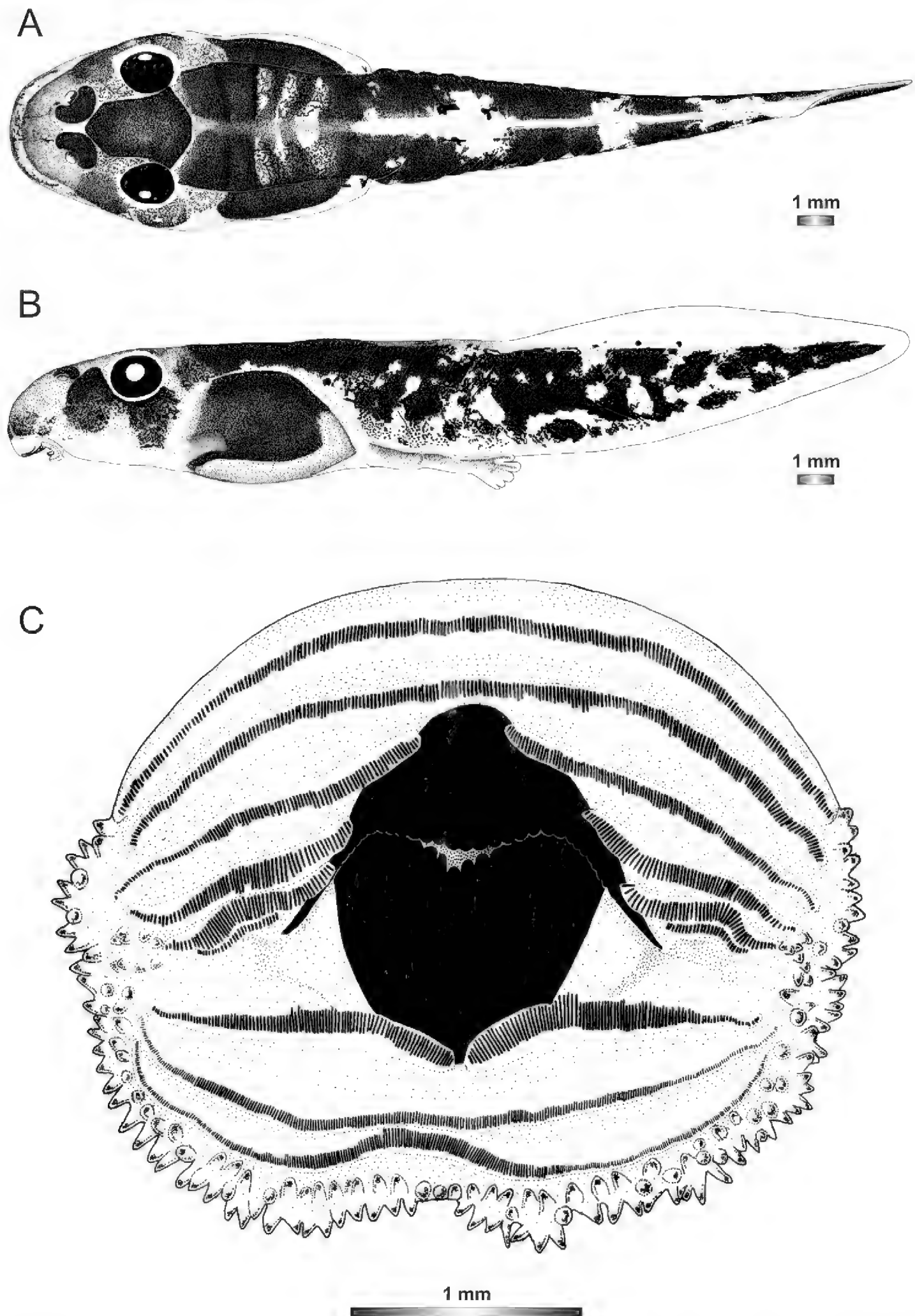


**Figure 1.** Coloration in life of strongly rheophilous tadpoles of *Boophis* (dorsal, lateral and ventral views): **A** *B. andohahela* (T 09/273-ZSM 282/2009) **B** *B. ankaratra* (ZCMV 4917-ZSM 876/2007) **C** *B. schuboeae* (T 09/980-743/2008) **D** *B. sibilans* (ZCMV 11548 - to be catalogued in ZSM) **E** *B. luciae* (ZCMV 11548-to be catalogued in ZSM) **F** *B. albipunctatus* (ZCMV 4946-ZSM 82/2008) **G** *B. mandraka* [Ca38] (ZCMV 4261-ZSM 456/2007) **H** *B. sambirano* [Ca47] (ZCMV 13105-ZSM 482/2010) **I** *B. sambirano* [Ca48] (ZCMV 13109-ZSM 486/2010) **J** *B. sambirano* [Ca49] (ZCMV 13155-ZSM 528/2010) **K** *B. sambirano* [Ca50] (ZCMV 13172-ZSM 545/2010) **L** *B. marojezensis* [Ca51] (ZCMV 13550-ZSM 721/2010) **M** *B. marojezensis* [Ca52] (ZCMV 13168-ZSM 541/2010) **N** *B. marojezensis* [Ca53] (ZCMV 13200-ZSM 573/2010).



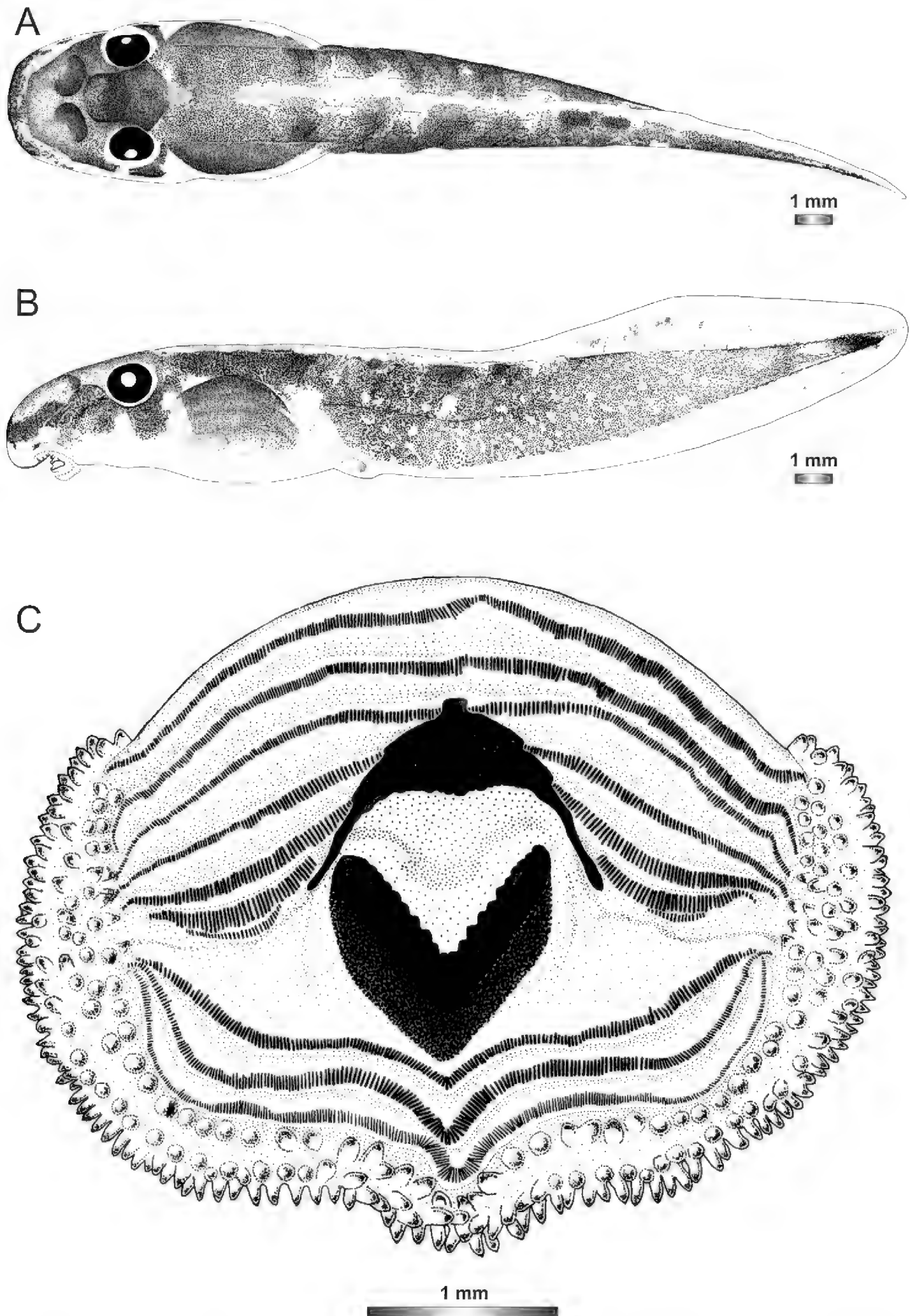
**Figure 2.** Pictures of the oral discs of the voucher specimens (stained with methylene blue for better visibility of morphological structures). Scale bars represent 1 mm.

*B. albipunctatus* tadpoles can be distinguished from those of *B. ankaratra* and *B. schuboeae* by the absence of the lateral bulge on the oral disc, the absence of the medial convexity on the upper sheath, the high number of papillae, and the LTRF 7(5–7)/3,

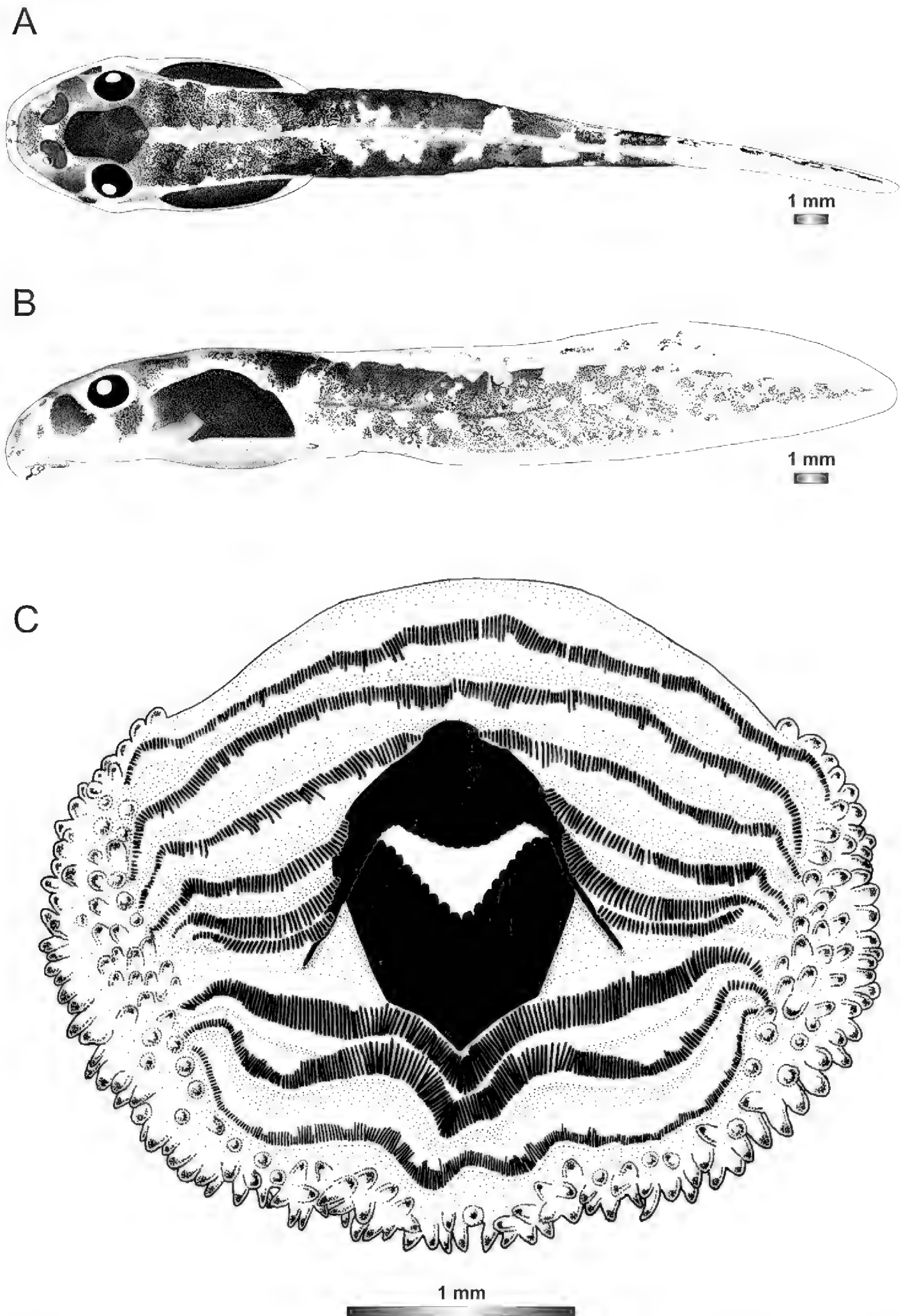


**Figure 3.** Drawings of the preserved DNA voucher tadpole of *Boophis englaenderi* (FGZC 2244-ZSM 623/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.

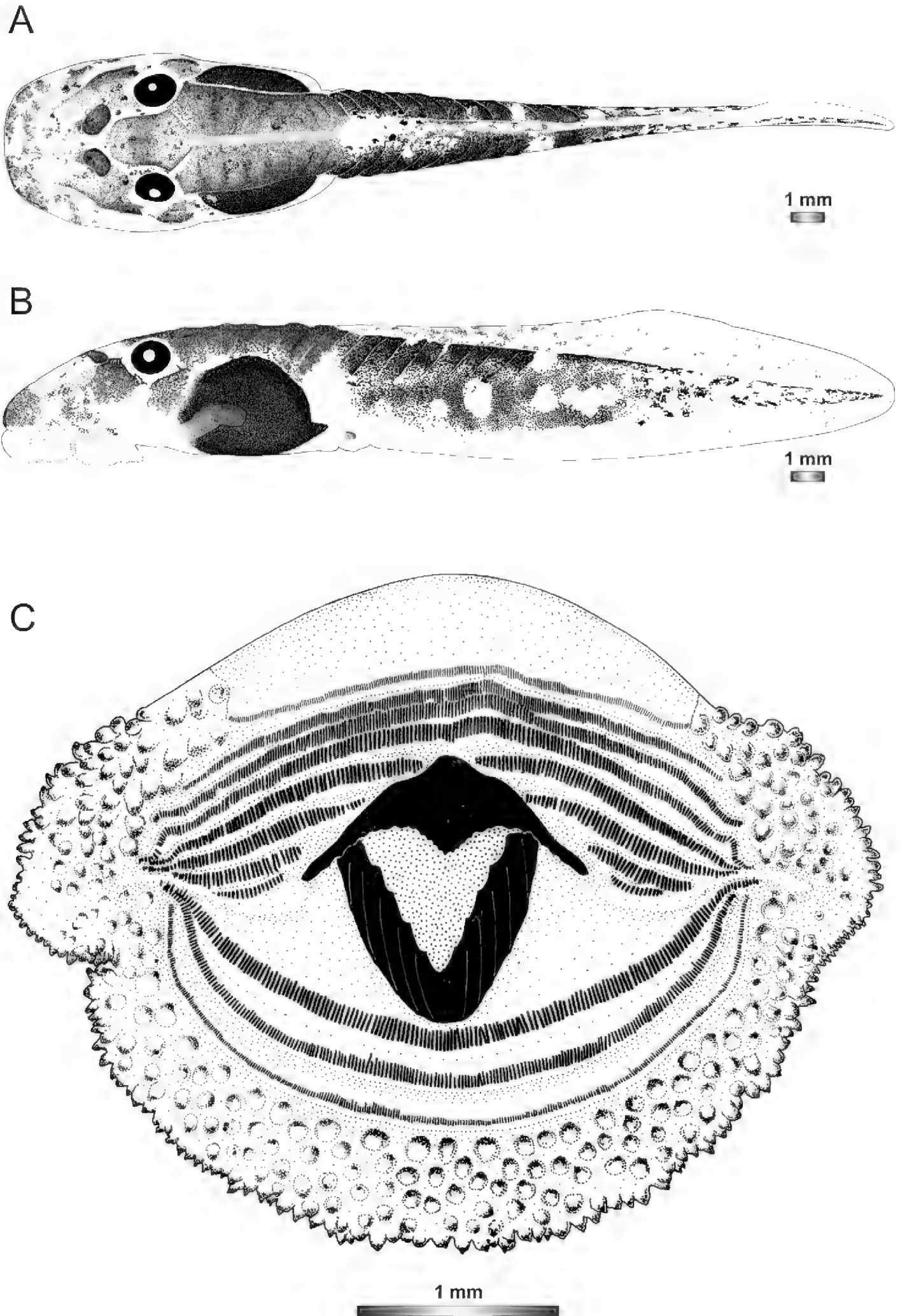




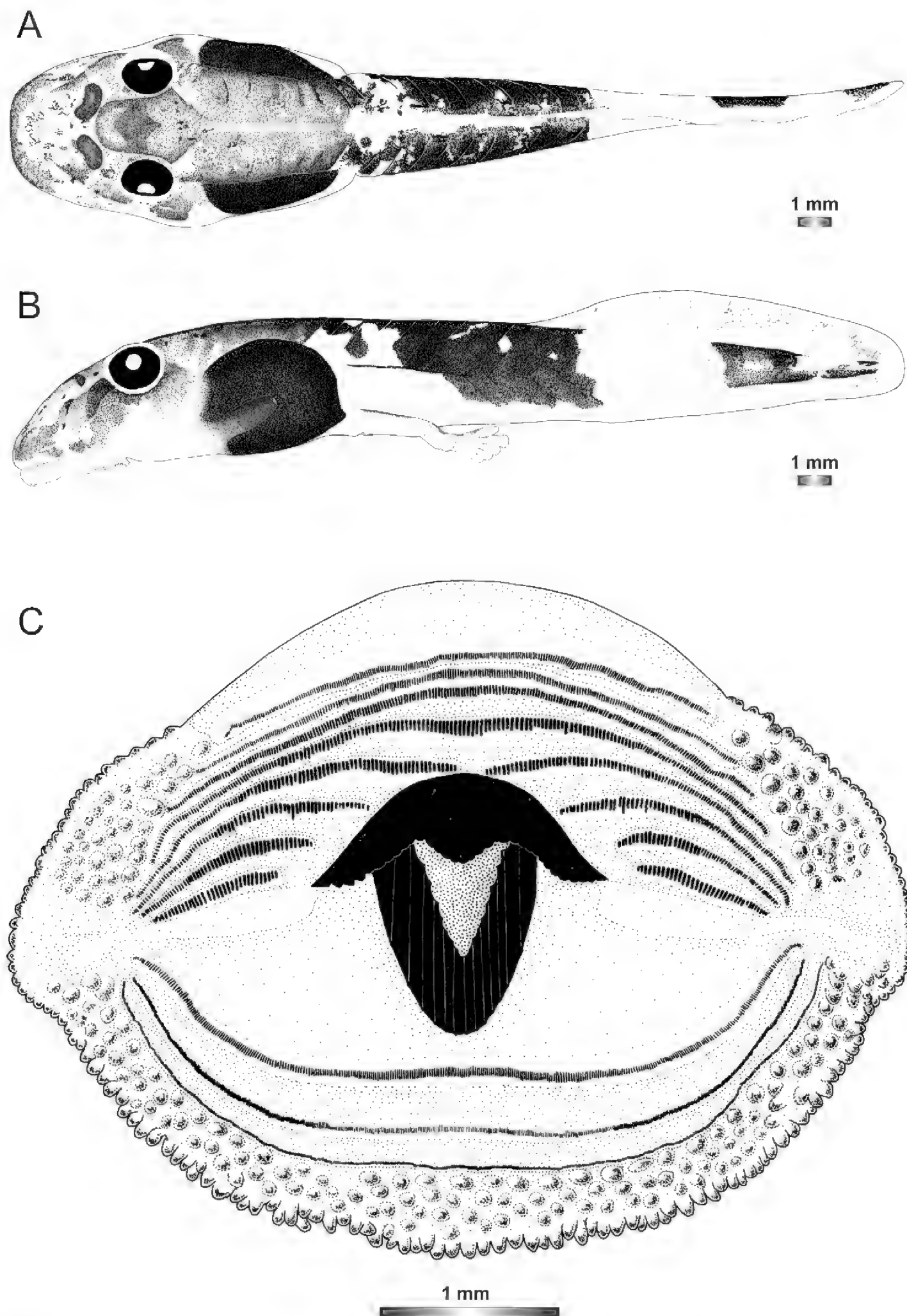
**Figure 4.** Drawings of the preserved DNA voucher tadpole of *Boophis englaenderi* [Ca23] (FGZC 2957-ZSM 1632/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 5.** Drawings of the preserved DNA voucher tadpole of *Boophis andohahela* (T 428-ZSM 998/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

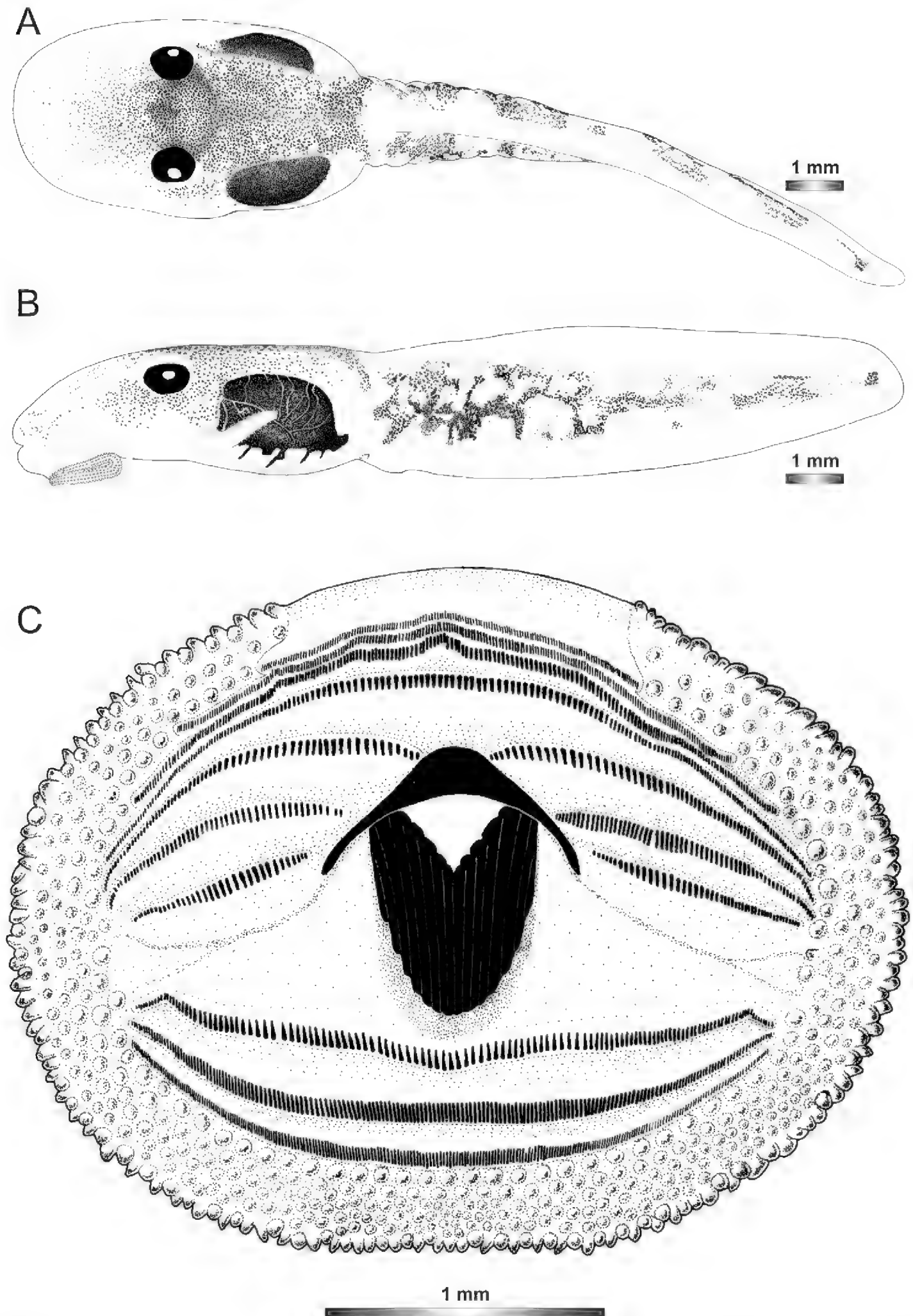


**Figure 6.** Drawings of the preserved DNA voucher tadpole of *Boophis ankaratra* (ZCMV 4917-ZSM 876/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 7.** Drawings of the preserved DNA voucher tadpole of *Boophis schuboeae* (FG/MV 2003.1800-ZSM 978/2004): **A** Dorsal view **B** Lateral view **C** Oral disc.





**Figure 8.** Drawings of the preserved DNA voucher tadpole of *Boophis albipunctatus* (ZCMV 4946-ZSM 82/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.



but they share the ribbed pattern, the U-shape, and the partially hidden state of the lower jaw sheath. These tadpoles are also characterized by their less pigmented state in preservative which makes them easy to identify. The absence of silver pigment covering the heart in life is also typical for these tadpoles.

### ***Boophis sibilans* Glaw & Thiesmeier, 1993**

Morphological data were assessed in one tadpole (Figures 2 and 9) in developmental stage 29 (field number FGZC 2956, ZSM 1631/2007, BL 11 mm, TL 26 mm, Genbank accession number JQ518194) from Marojejy National Park. The 16S rDNA sequence of this specimen is 99.4% identical to a reference sequence of a *B. sibilans* adult specimen (accession AY341718) from Andasibe. Two other voucher tadpoles have similar morphological characteristics.

*B. sibilans* tadpoles have the same oral disc feature (absence of lateral bulge, LTRF) as *B. albipunctatus*, except for a lower number of submarginal papillae and a V-shaped lower sheath. These tadpoles are characterized by their rather long tail (up to 200% of BL) and their unique tail pattern which is composed of dark spots separated by a clear unpigmented area. The inner part of the spots is usually free from pigment (Figure 1).

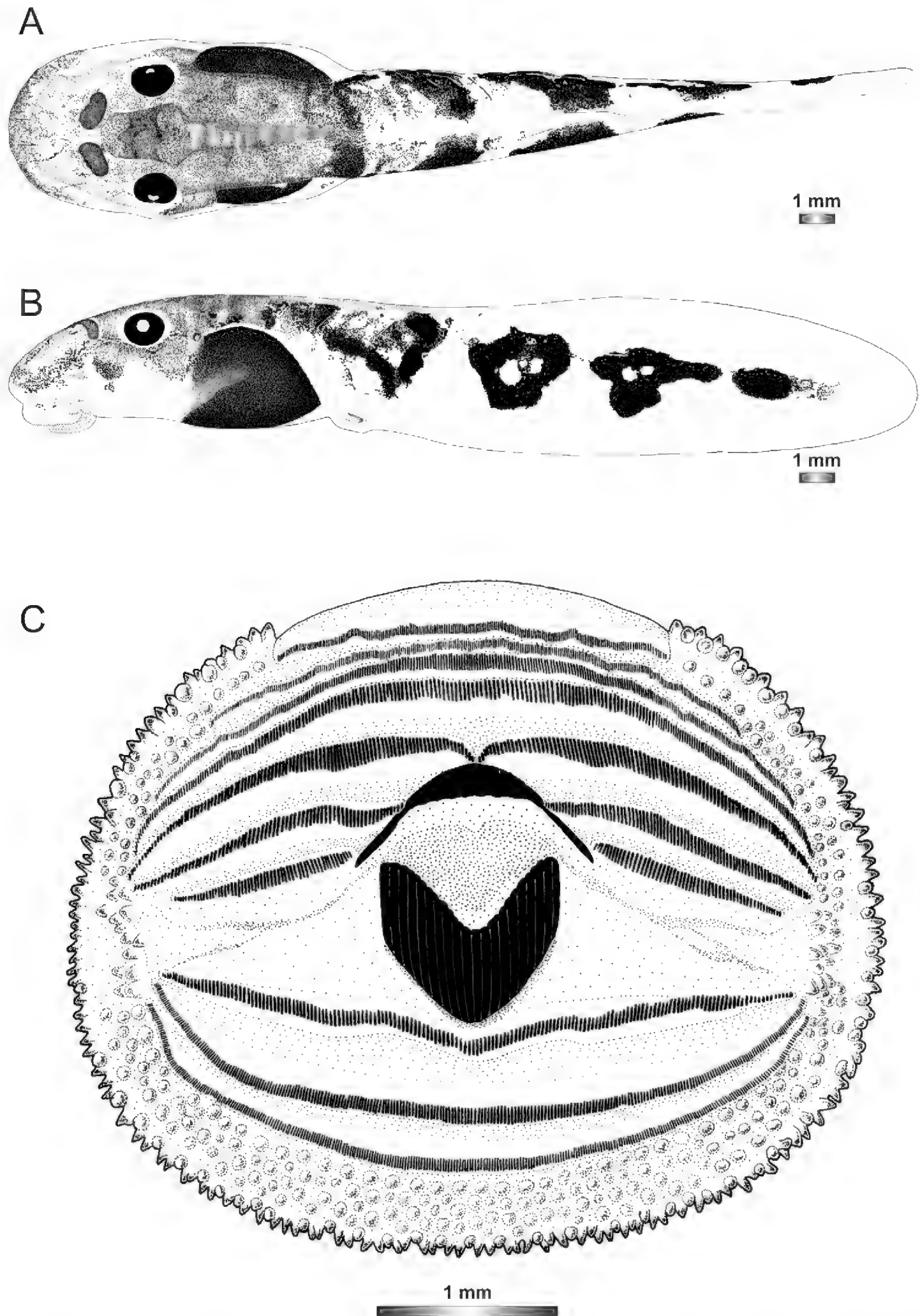
### ***Boophis luciae* Glaw, Köhler, de la Riva, Vieites & Vences, 2010**

Morphological data were assessed in one tadpole (Figures 2 and 10) in developmental stage 36 (field number ZCMV 5146, ZSM 730/2007, BL 10.4 mm, TL 22.2 mm, Genbank accession number GU975069) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of a *B. luciae* adult specimen (accession AY848444) from the same locality. Ten other voucher tadpoles are morphologically very similar to the described voucher specimen.

The tadpoles of *B. luciae* are similar to those of *B. sibilans* by their oral disc structure and the general external pattern except that they have a rather short tail. They can be characterized by the state of the spots on the tail musculature which are connected to each other (Figure 1).

### ***Boophis mandraka* group**

This group is characterized by tadpoles having an enlarged oral disc without lateral emargination and ventral gap of papillae. The dorsal gap of papillae is narrow to very narrow, and the lateral area where the oral disc folds is free of submarginal papillae. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the



**Figure 9.** Drawings of the preserved DNA voucher tadpole of *Boophis sibilans* (FGZC 2956-ZSM 1631/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

entire margin is free from the snout. The upper labium has always five uninterrupted and three interrupted keratodont rows, and the three lower rows are always uninterrupted giving a unique LTRF 8(6–8)/3. The upper sheath is always absent. The lower sheaths are moderately strong and completely keratinized, U-shaped, ribed, and higher than wide. Dorsolateral glands are present.

### ***B. sambirano* Vences & Glaw, 2005**

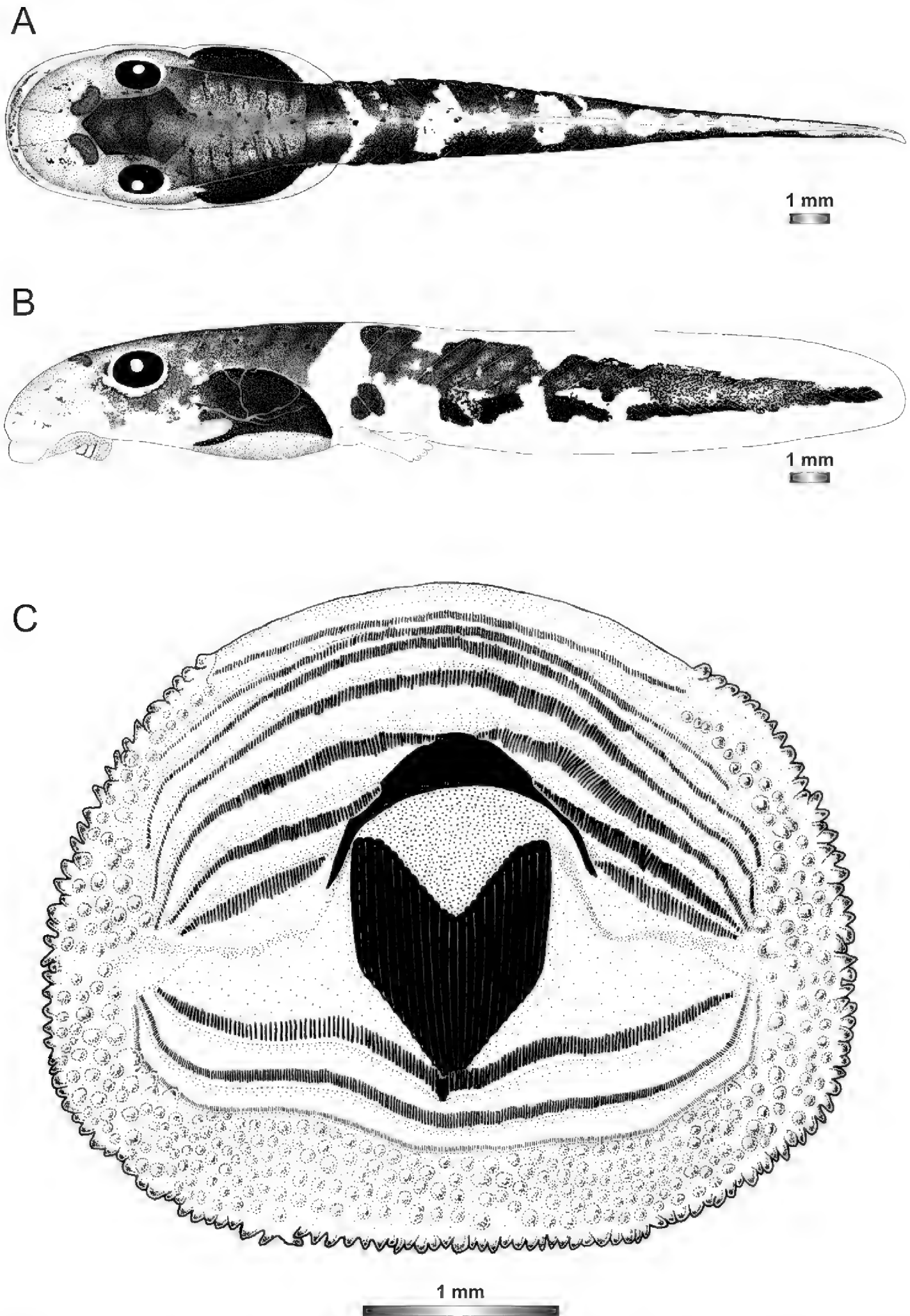
Morphological data were assessed in one tadpole (Figures 2 and 11) in developmental stage 25 (field number FG/MV 2002.1902, ZSM 672/2004, BL 6.5 mm, TL 12.7 mm, Genbank accession number EU717861) from a site locally named “Camp Norbert” in Manongarivo Special Reserve. The 16S rDNA sequence of this specimen is 96% identical to a reference sequence of *B. sambirano* adult specimen (accession AY848544), and because of this 4% difference its identity and belonging to the “true” *B. sambirano* needs further confirmation. Since this specimen was collected next to the type locality of *B. sambirano* in Manongarivo, following a parsimonious approach we here assign it to this species, although the large numbers of distinct lineages in *B. sambirano* make it likely that yet another candidate species of this complex occurs in Manongarivo. Many non-voucher specimens of the same series present morphological similarities to the voucher specimen.

*B. sambirano* tadpoles are easy to distinguish from all other tadpoles described above by the state of their oral disc which has no upper jaw sheath, a short keratodont row  $A_1$ , and a narrow dorsal gap of papillae. The absence of submarginal papillae on the lateral area where the oral disc folds is shared with *B. schuboeae*. The tadpoles of this species are also characterized by the extension of an obvious lateral transparent area of the body wall only on the anterior 2/3 of the body, but not surrounding the whole body like in other tadpoles.

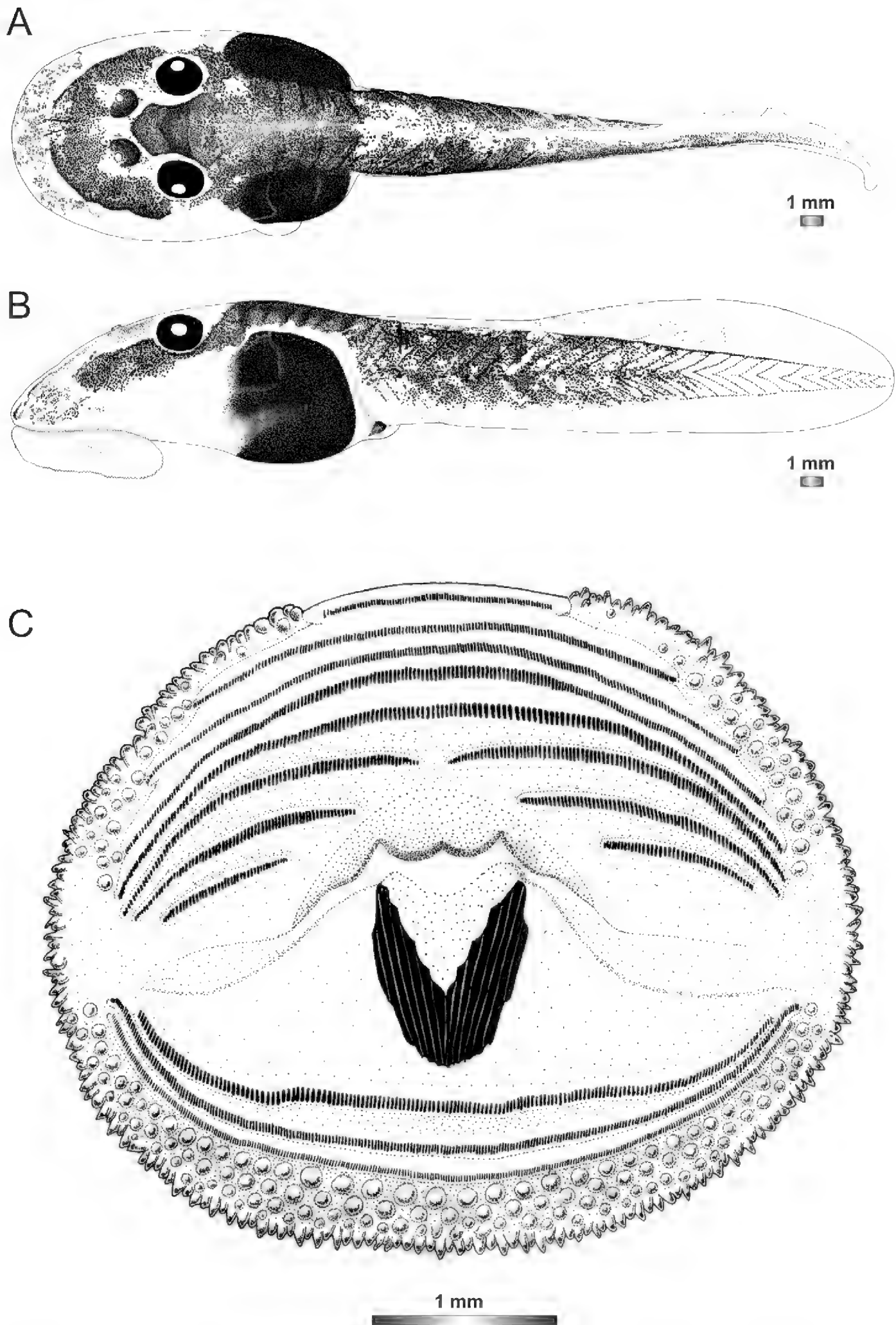
### ***Boophis mandraka* [Ca38 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 12) in developmental stage 26 (field number ZCMV 4261, ZSM 456/2007, BL 7.6 mm, TL 15.8 mm, Genbank accession number FJ559153) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 93.3 % identical to a reference sequence of a *Boophis sambirano* adult specimen (accession EU717863) from Manongarivo Special Reserve.

The single tadpole of this candidate species has a similar oral disc structure to *B. sambirano* except that it has a slightly wider dorsal gap of papillae (DG/ODW 39% vs. 34%). The typical coloration, yellowish in life (Figure 1) and whitish in preservative and the good visibility of the 10 (5 right and 5 left) dorsolateral glands allow its distinction from other tadpoles.

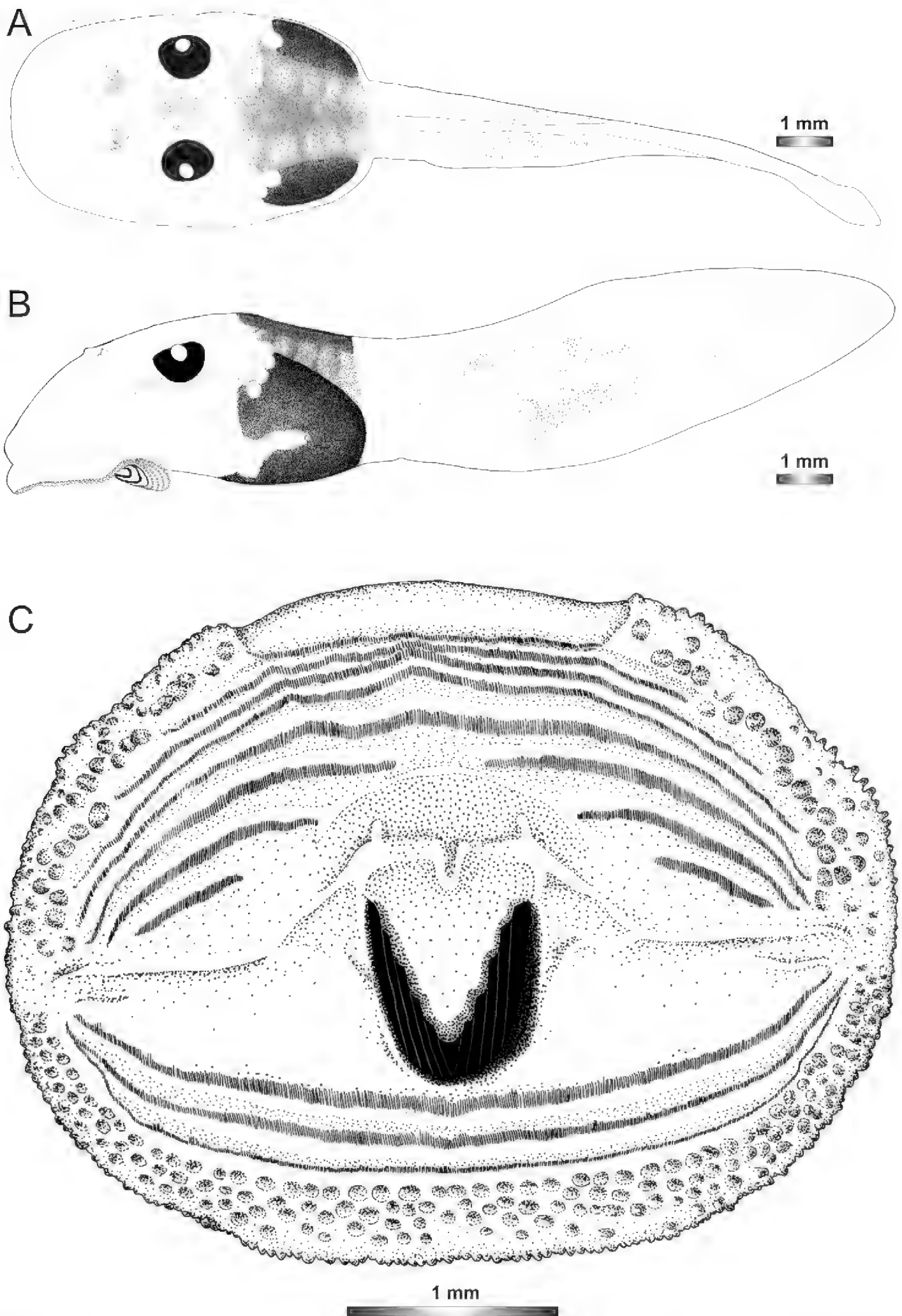


**Figure 10.** Drawings of the preserved DNA voucher tadpole of *Boophis luciae* (ZCMV 5146-ZSM 730/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 11.** Drawings of the preserved DNA voucher tadpole of *Boophis sambirano* (FG/MV 2002.1904-ZSM 678/2004): **A** Dorsal view **B** Lateral view **C** Oral disc.





**Figure 12.** Drawings of the preserved DNA voucher tadpole of *B. mandraka* [Ca38] (ZCMV 4261-ZSM 456/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

***Boophis mandraka* [Ca46 JQ518195]**

Morphological data were assessed in one tadpole (Figures 2 and 13) in developmental stage 25 (field number ZCMV 3479, ZSM 1784/2007, BL 6.8 mm, TL 14.3 mm, Genbank accession number JQ518195) from An'Ala. The 16S rDNA sequence of this specimen is 90.4 % identical to a reference sequence of *Boophis* sp. aff. *mandraka* adult specimen (accession AY848542) from Ilampy.

The oral disc of the single tadpole of this candidate species is similar to those of *B. sambirano* and *B. mandraka* [Ca38] except that it has the narrowest dorsal gap of papillae with DG 14% of ODW and the shortest  $A_1$  with 21% of ODW. Within the *Boophis mandraka* group tadpoles, it has also the lowest number of papillae. The external morphology of the single tadpole of this candidate species is similar to that of tadpoles of *B. sambirano*, except that the ratio RN/NP is much higher (194 *vs.* 125) and the pigmentation pattern is slightly different.

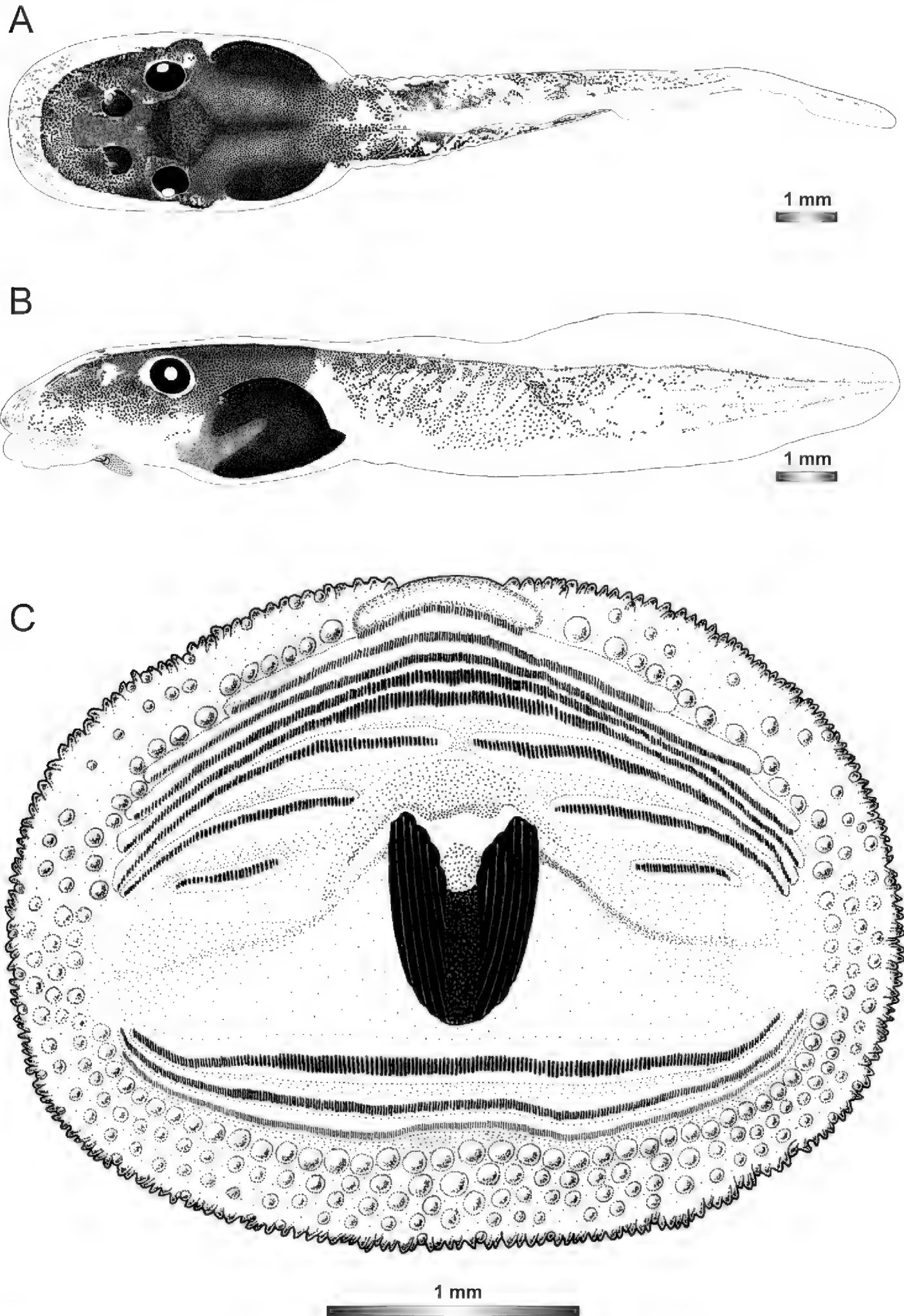
***Boophis sambirano* [Ca47 JQ518203]**

Morphological data were assessed in one tadpole (Figures 2 and 14) in developmental stage 27 (field number ZCMV 13105, ZSM 482/2010, BL 13.5 mm, TL 27.1 mm, Genbank accession number JQ518203) from Anjingo river (bridge 57 km from Antsohihy to Bealanana). The 16S rDNA sequence of this specimen is 97% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Two other voucher tadpoles are morphologically very similar to the described voucher specimen.

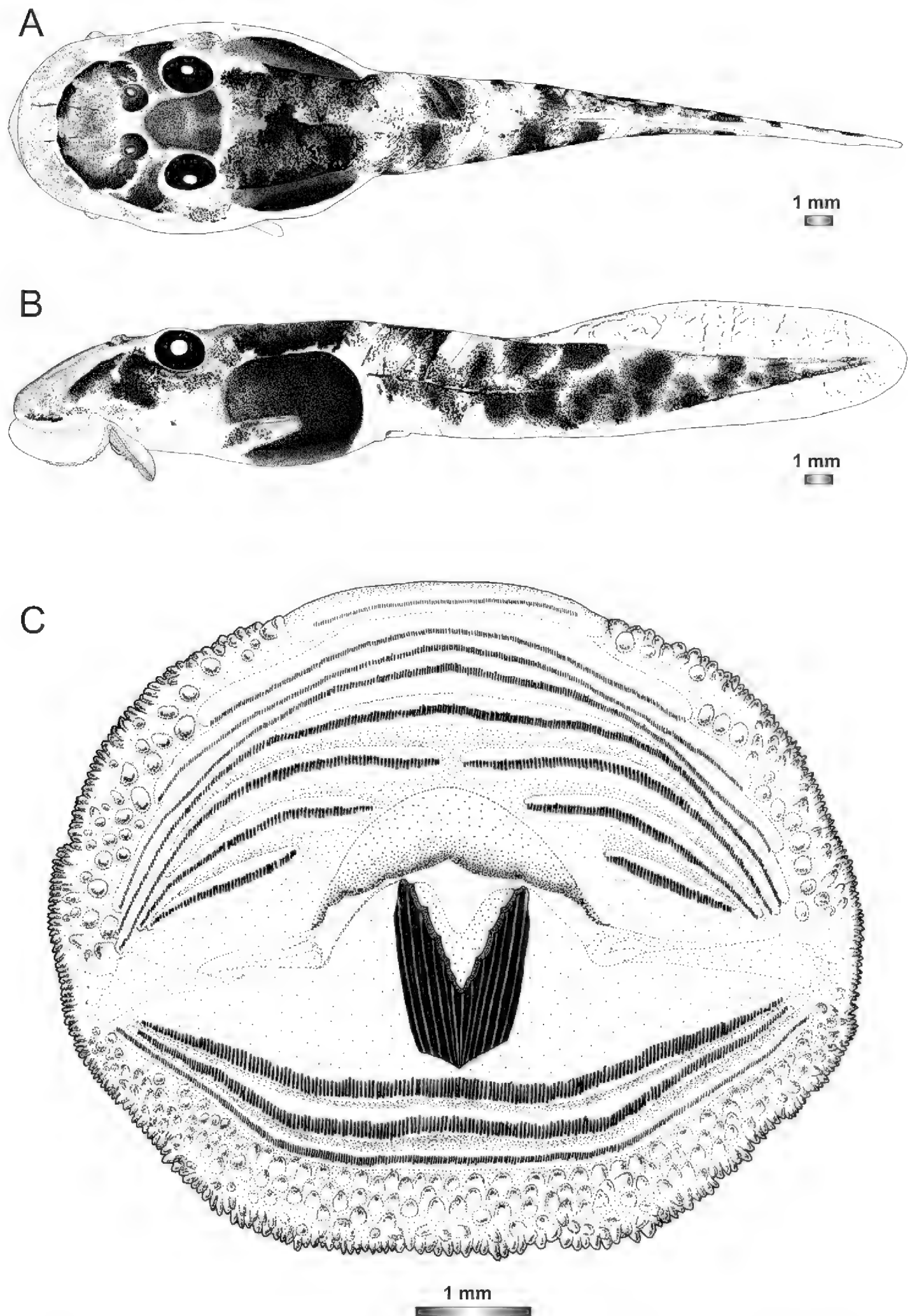
The tadpoles assigned to this candidate species have a similar oral disc structure as *B. sambirano* except that they have a higher number of marginal papillae (377 *vs.* 248) and of keratodonts on  $A_3$  (1193 *vs.* 740). These tadpoles have a rather large size in comparison to others of the *B. mandraka* group, and their pigmentation pattern distinguishes them also. Their tail musculature is covered by dissipated distinct patches following mainly the lateral tail vein and the myosepta on the anterior half of the tail musculature, and irregularly dispersed on the posterior half (Figure 1), whereas it is just covered by dense spots on the anterior half in *B. sambirano* tadpoles. The dorsal fin of these tadpoles begins usually on the anterior 1/5 of the tail musculature, *vs.* beginning more or less at the dorsal body-tail junction in *B. sambirano*.

***Boophis sambirano* [Ca48 JQ518205]**

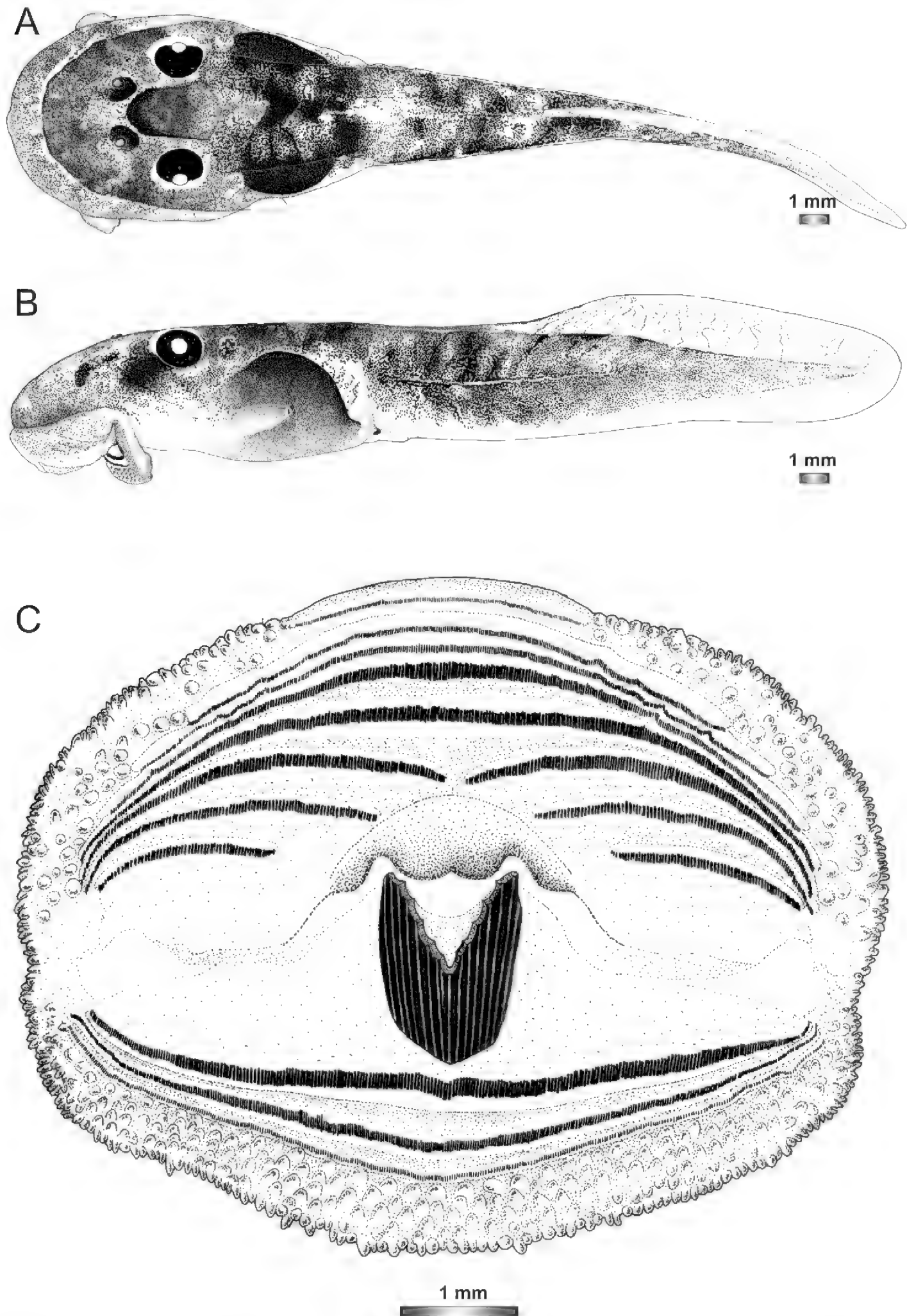
Morphological data were assessed in one tadpole (Figures 2 and 15) in developmental stage 27 (field number ZCMV 13109, ZSM 485/2010, BL 12.7 mm, TL 24.7



**Figure 13.** Drawings of the preserved DNA voucher tadpole of *B. mandraka* [Ca46] (ZCMV 3479-ZSM 1784/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 14.** Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca47] (ZCMV 13105-ZSM 482/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 15.** Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca48] (ZCMV 13109-ZSM 485/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.



mm, Genbank accession number JQ518205) from Anjingo river (bridge at 57 km on the road from Antsohihy to Bealanana). The 16S rDNA sequence of this specimen was 94% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Two other voucher tadpoles are very similar to the described voucher specimen.

The tadpoles assigned to this candidate species have a similar oral disc as *B. sambirano* and *B. sambirano* [Ca47]. The higher number of marginal papillae (336) and of keratodonts on  $A_3$  (1052) differentiate these tadpoles from those of *B. sambirano* but are similar to *B. sambirano* [Ca47]. The ovoidal body form in dorsal view and the pigmentation pattern – variegated spots on the body and less coalesced spots on the tail musculature (Figure 1) – differentiate these tadpoles from those of *B. sambirano* [Ca47]. The beginning of the dorsal fin on the anterior 1/5 of the tail musculature is similar to that of *B. sambirano* [Ca47] but different from *B. sambirano*.

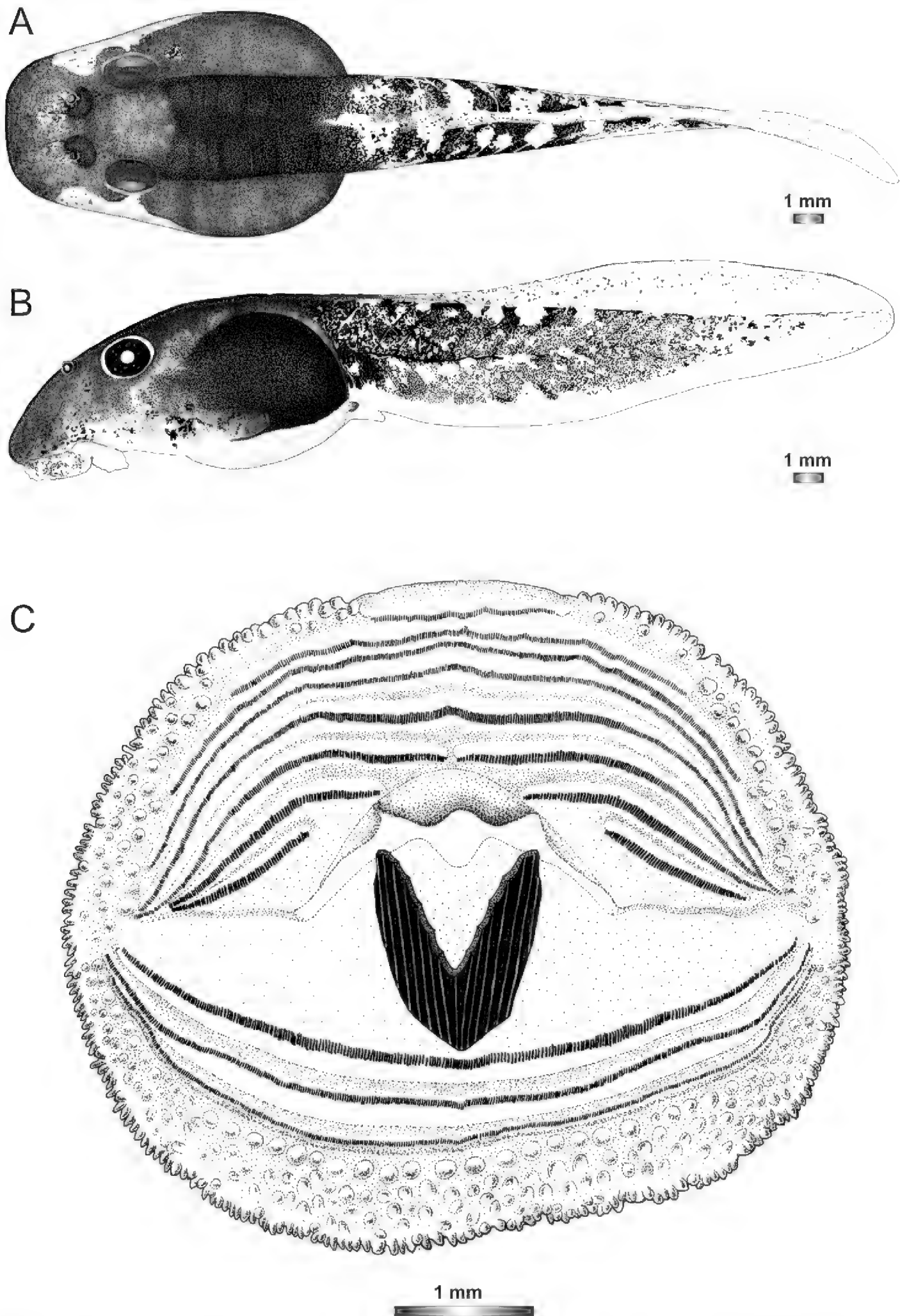
### ***Boophis sambirano* [Ca49 JQ518208]**

Morphological data were assessed in one tadpole (Figures 2 and 16) in developmental stage 27 (field number ZCMV 13155, ZSM 528/2010, BL 11.7 mm, TL 26.7 mm, Genbank accession number JQ518208) from Ankijagna Lagnana. The 16S rDNA sequence of this specimen is 94.1% identical to a reference sequence of *Boophis sambirano* tadpoles (accession EU717861) from Manongarivo Special Reserve. Three other voucher specimens and many non-voucher specimens of the same series are morphologically very similar to the described specimen.

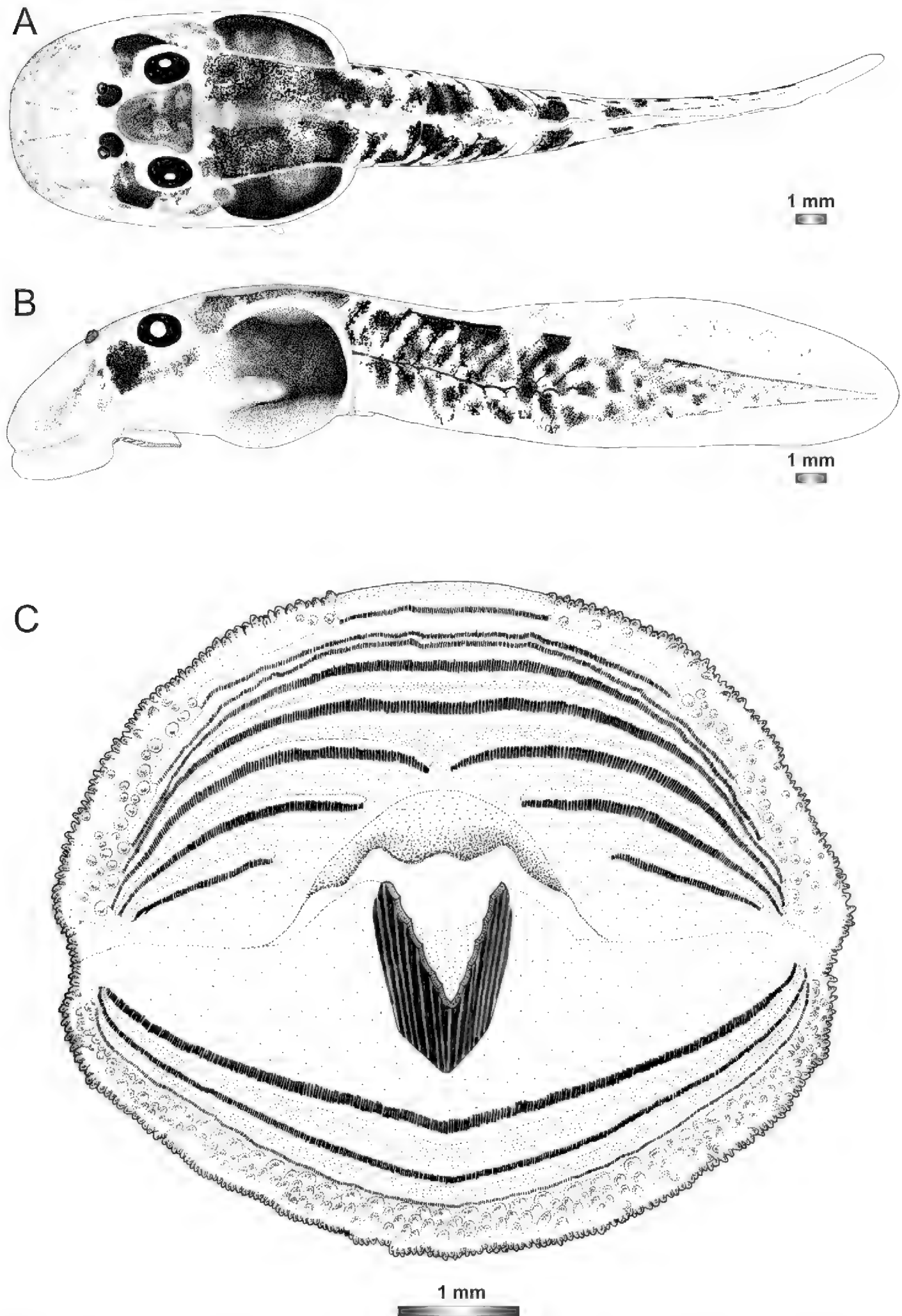
The oral disc of the tadpoles assigned to this candidate species is the typical one of the *B. mandraka* group, characterized by a narrow dorsal gap of papillae (DG 23% of ODW) which is here wider than in *B. mandraka* [Ca46] but smaller than in the other tadpoles, and the short keratodont row  $A_1$  which is similar to that of *B. mandraka* [Ca46] tadpoles. The number of papillae is similar to that of *B. sambirano* and *Boophis mandraka* [Ca38]. These tadpoles can be easily distinguished from all *B. sambirano*-like tadpoles by their particular pigmentation pattern which is uniformly dark (Figure 1), by the non visibility of the lateral transparent area of the body wall, the ovoidal form of the body in dorsal view, and the eye position between the anterior 3/10 and 4/10 of the body.

### ***Boophis sambirano* [Ca50 JQ518211]**

Morphological data were assessed in one tadpole (Figures 2 and 17) in developmental stage 27 (field number ZCMV 13172, ZSM 545/2010, BL 11.7 mm, TL 25.7 mm, Genbank accession number JQ518211) from Ambinanitelo. The 16S rDNA sequence of this specimen is 94.9% identical to a reference sequence of *Boophis sambirano* tad-



**Figure 16.** Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca49] (ZCMV 13155-ZSM 528/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 17.** Drawings of the preserved DNA voucher tadpole of *B. sambirano* [Ca50] (ZCMV 13172-ZSM 545/2010): **A** Dorsal view **B** Lateral view; **C** Oral disc.

poles (accession EU717861) from Manongarivo Special Reserve. Three other voucher tadpoles are morphologically very similar to the described voucher specimen.

The oral disc of the tadpoles of this candidate species is similar to that of other *B. mandraka* group species. The tadpoles belonging to this candidate species have an elliptical body form in dorsal view but differ from those of *B. sambirano* [Ca49] by their pigmentation pattern. The presence of a lateral transparent area of the body wall surrounding the anterior 2/3 of the body is similar to those of *B. sambirano*, but the absence of contrasted integumental patches limiting the transparent body wall area surrounding the snout is a difference to *B. sambirano*, *Boophis sambirano* [Ca47], and *Boophis sambirano* [Ca48]. The tadpoles of this candidate species can thus be distinguished from those of other candidate species close to *B. sambirano* mainly by their coloration pattern (Figure 1).

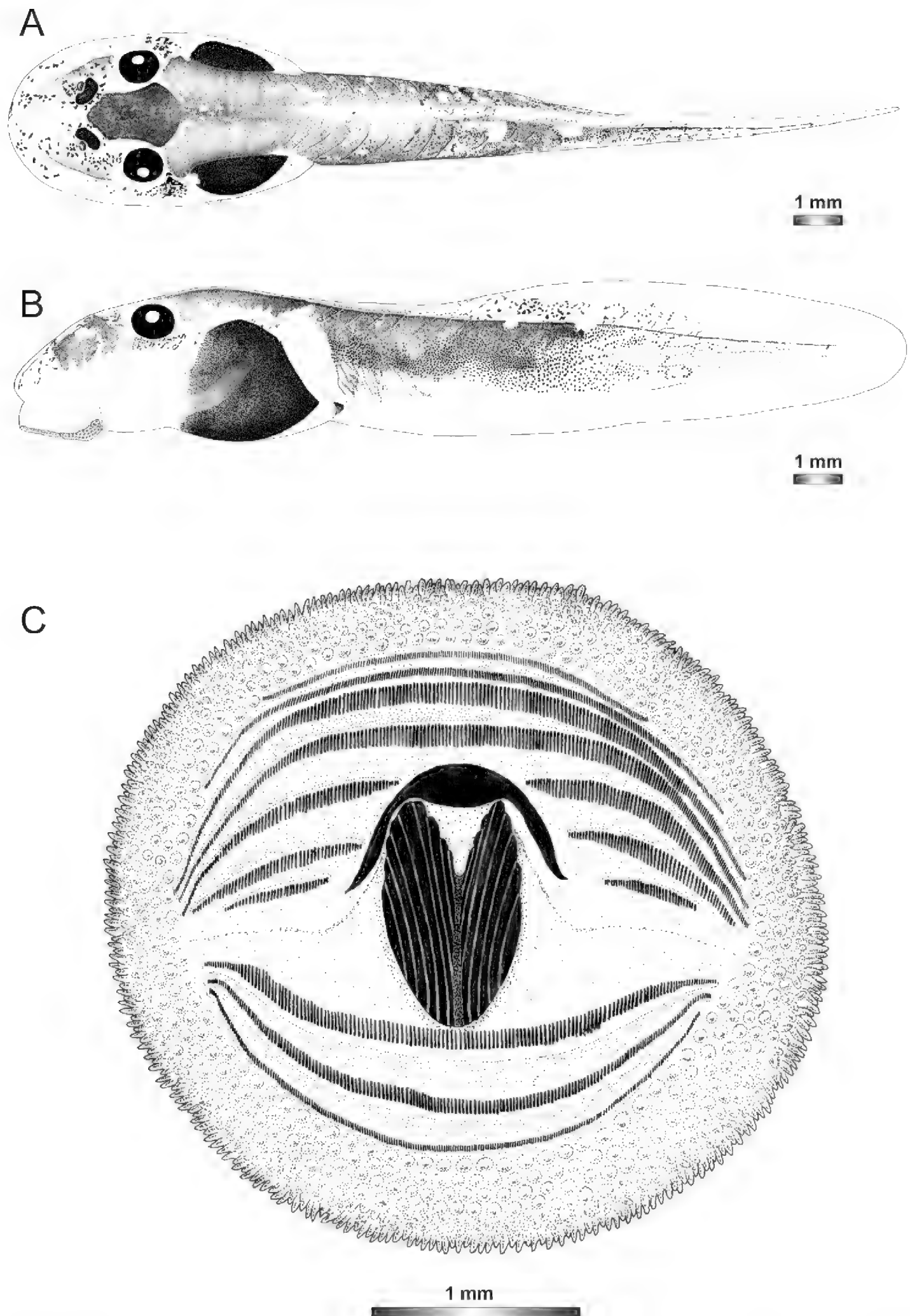
### ***Boophis majori* group**

This group is heterogeneous in larval morphology and probably non monophyletic (e.g., Schmidt et al. 2008; Randrianiana et al. 2009a). The rheophilous tadpoles in this group with an enlarged oral disc are further characterized by the absence of a lateral emargination, and absence of dorsal and ventral gaps of papillae. The submarginal papillae are complete. The anterior margin of the oral disc is separated by a deep crevice to the snout; i.e., the entire margin is free from the snout. LTRF 7(5–7)/3. The jaw sheaths are moderately strong and completely keratinized. The upper sheath always lacks a medial convexity. The lower sheath is U-shaped, ribbed, and higher than wide. Dorsolateral glands are present.

### ***Boophis marojezensis* Glaw & Vences, 1994**

Morphological data were assessed in one tadpole (Figures 2 and 18) in developmental stage 27 (field number FGZC 2277, ZSM 1528/2007, BL 7.1 mm, TL 18.3 mm, Genbank accession number JQ518196), from Marojejy National Park. The 16S rDNA sequence of this specimen is 99.8% identical to a reference sequence of a *B. marojezensis* adult specimen (accession FJ559127) from the same locality. Three other voucher tadpoles are morphologically very similar to the described voucher specimen.

The tadpoles of this species are easily to distinguish from those belonging to other species groups (as described above) by the general structure of their oral disc which has no dorsal gap of papillae, and a LTRF of 7(5–7)/3. These tadpoles are also characterized by the highest number of submarginal papillae in *Boophis*, with 290 marginal and 606 submarginal papillae. The lateral transparent area of the body wall area is visible and the dorsolateral gland is obvious. The tail muscle is spotted and the spots fused



**Figure 18.** Drawings of the preserved DNA voucher tadpole of *B. marojezensis* (FGZC 2277-ZSM 1528/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



to form patches mainly on the upper half of tail musculature, the density of the spots diminishes toward the tail tip. The posterior 1/3 of the tail has few pigments.

***Boophis marojezensis* [Ca25 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 19) in developmental stage 29 (field number FGZC 2929, ZSM 1611/2007, BL 7.8 mm, TL 18.5 mm, Genbank accession number FJ559146), from Marojejy National Park. The 16S rDNA sequence of this specimen is 97% identical to a reference sequence of *B. marojezensis* adult specimen (accession AY848596) from Vohidrazana, and less similar to other tadpoles from Marojejy. Two non-voucher specimens from the same series have the particular caudal pattern present in the voucher specimen.

Tadpoles assigned to this candidate species have the same oral disc structure as those of *B. marojezensis*, but with a lower number of papillae (222 marginal and 315 submarginal). The presence of seven more or less rounded patches formed by condensation of spots on the posterior half of the tail musculature of these tadpoles is a further useful character to differentiate them from those of *B. marojezensis*.

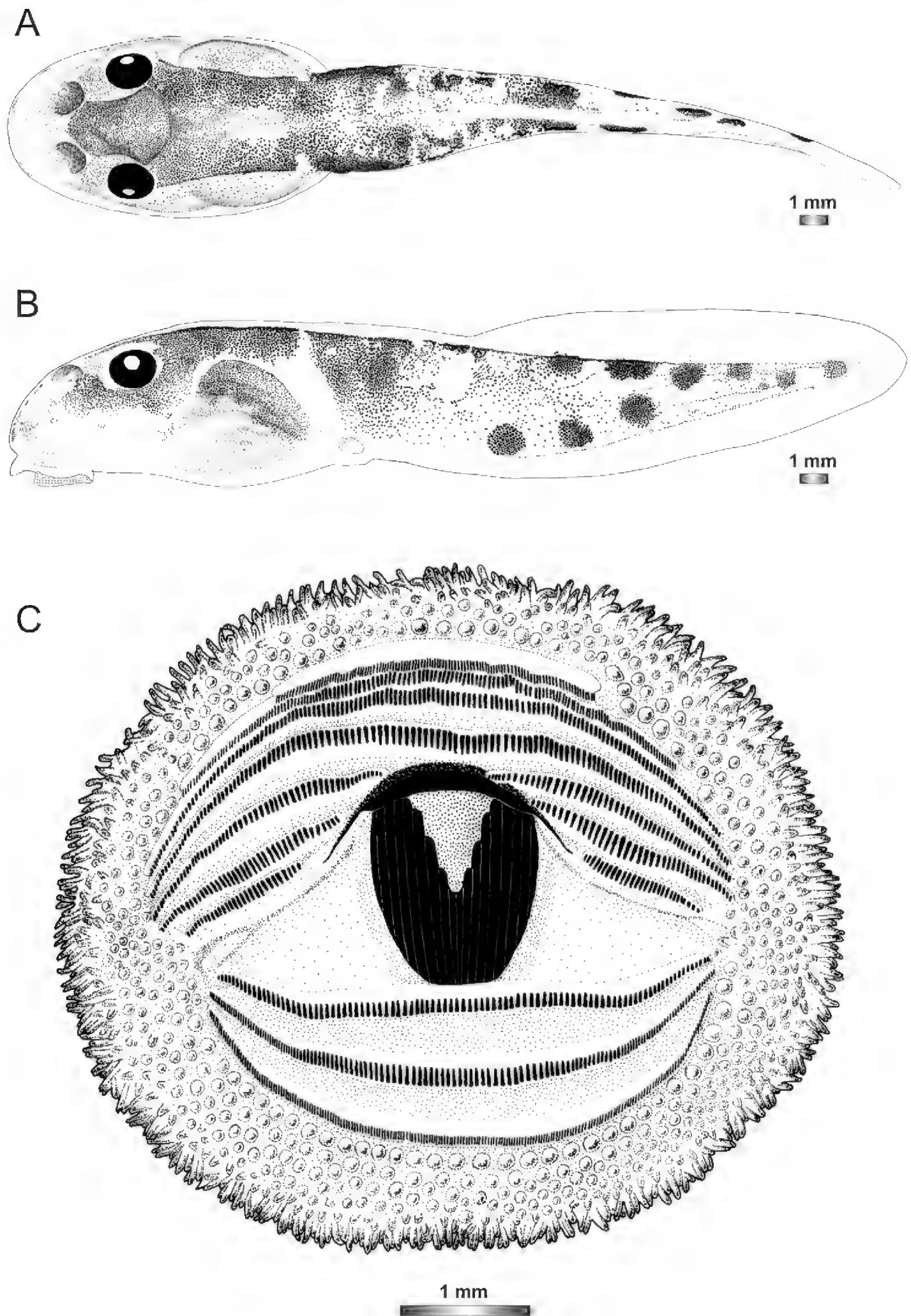
***Boophis marojezensis* [Ca26 Vieites et al. 2009]**

Morphological data were assessed in one tadpole (Figures 2 and 20) in developmental stage 29 (field number FGZC 2930, ZSM 1612/2007, BL 8.8 mm, TL 20.6 mm, Genbank accession number JQ518197), from Marojejy National Park. The 16S rDNA sequence of this specimen is 96.6% identical to a reference sequence of a *B. marojezensis* adult specimen (accession AY848595) from Tsaratanana.

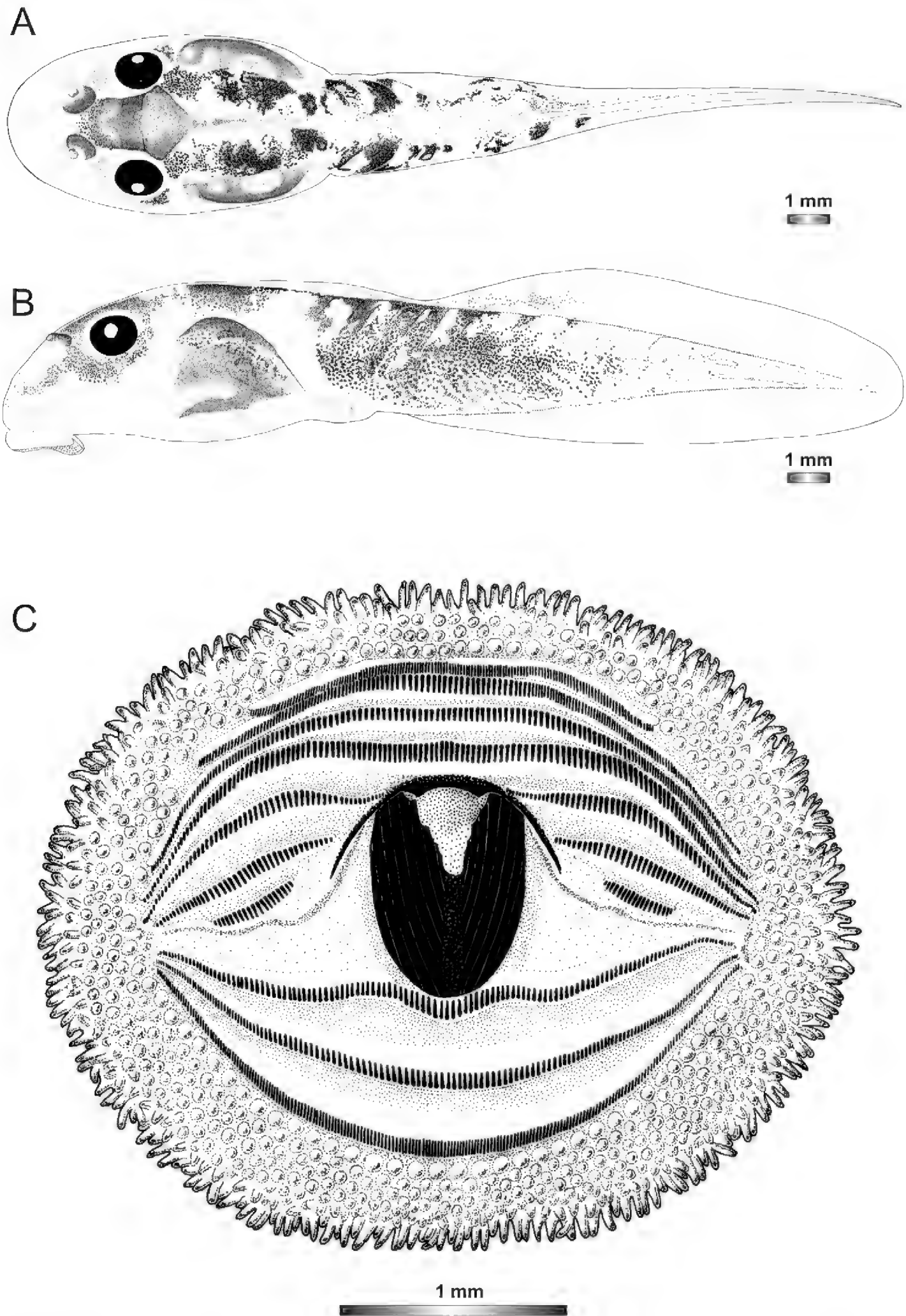
The single tadpole belonging to this candidate species has the typical *marojezensis*-like oral disc structure with 234 marginal and 430 submarginal papillae. It has almost the same pigmentation pattern as *B. marojezensis*, but the patches are more striking on the upper limit of tail musculature. It is differentiated from *B. marojezensis* [Ca25] by the absence of distinct patches on the tail musculature.

***Boophis marojezensis* [Ca51 JQ518198]**

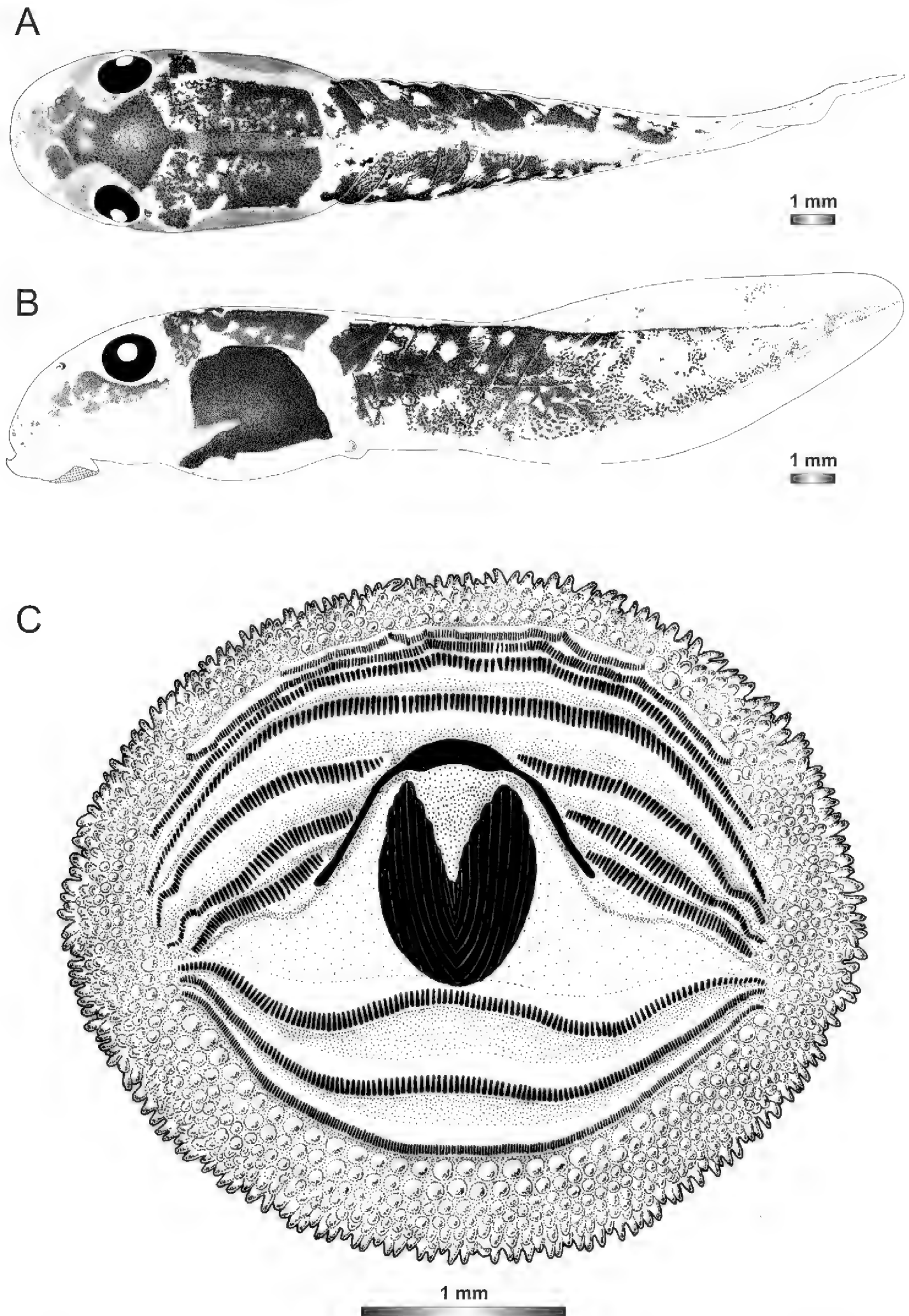
Morphological data were assessed in one tadpole (Figures 2 and 21) in developmental stage 25 (field number ZCMV 3691, ZSM 267/2008, BL 6 mm, TL 20 mm, Genbank accession number JQ518198) from Ranomafana National Park. The 16S rDNA sequence of this specimen is 99.7% identical to a reference sequence of a *B. marojezensis* adult specimen (accession AY848594) from Vohiparara (but with >5% divergence



**Figure 19.** Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca25] (FGZC 2929-ZSM 1611/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 20.** Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca26] (FGZC 2930-ZSM 1612/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 21.** Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca51] (ZCMV 3691-ZSM 267/2008): **A** Dorsal view **B** Lateral view **C** Oral disc.

to all other *B. marojezensis*-like forms). Twenty-one other tadpoles assigned to this candidate species reveal a similar morphological pattern and oral disc configuration as the described voucher specimen.

The tadpoles assigned to this candidate species have the typical *marojezensis*-like oral disc structure with 297 marginal and 309 submarginal papillae. They can be distinguished from the other *marojezensis*-like tadpoles by the absence of a lateral transparent area of the body wall area surrounding the body. They have also the widest inter-orbital distance (IOD) in the group, and they are also the only *marojezensis*-like tadpoles with eyes situated between the anterior 3/10 and 4/10 of the body. The tail muscle is covered by reticulations, mainly on the anterior half.

### ***Boophis marojezensis* [Ca52 JQ518215]**

Morphological data were assessed in one tadpole (Figures 2 and 22) in developmental stage 28 (field number ZCMV 13168, ZSM 541/2010, BL 10.5 mm, TL 26.1 mm, Genbank accession number JQ518215) from Ambinanitelo. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of an adult specimen assigned to *B. marojezensis* (accession AY848595) from Tsaratanana (but with >5% divergence to all other *B. marojezensis*-like forms). One other voucher specimen is morphologically very similar to the described one.

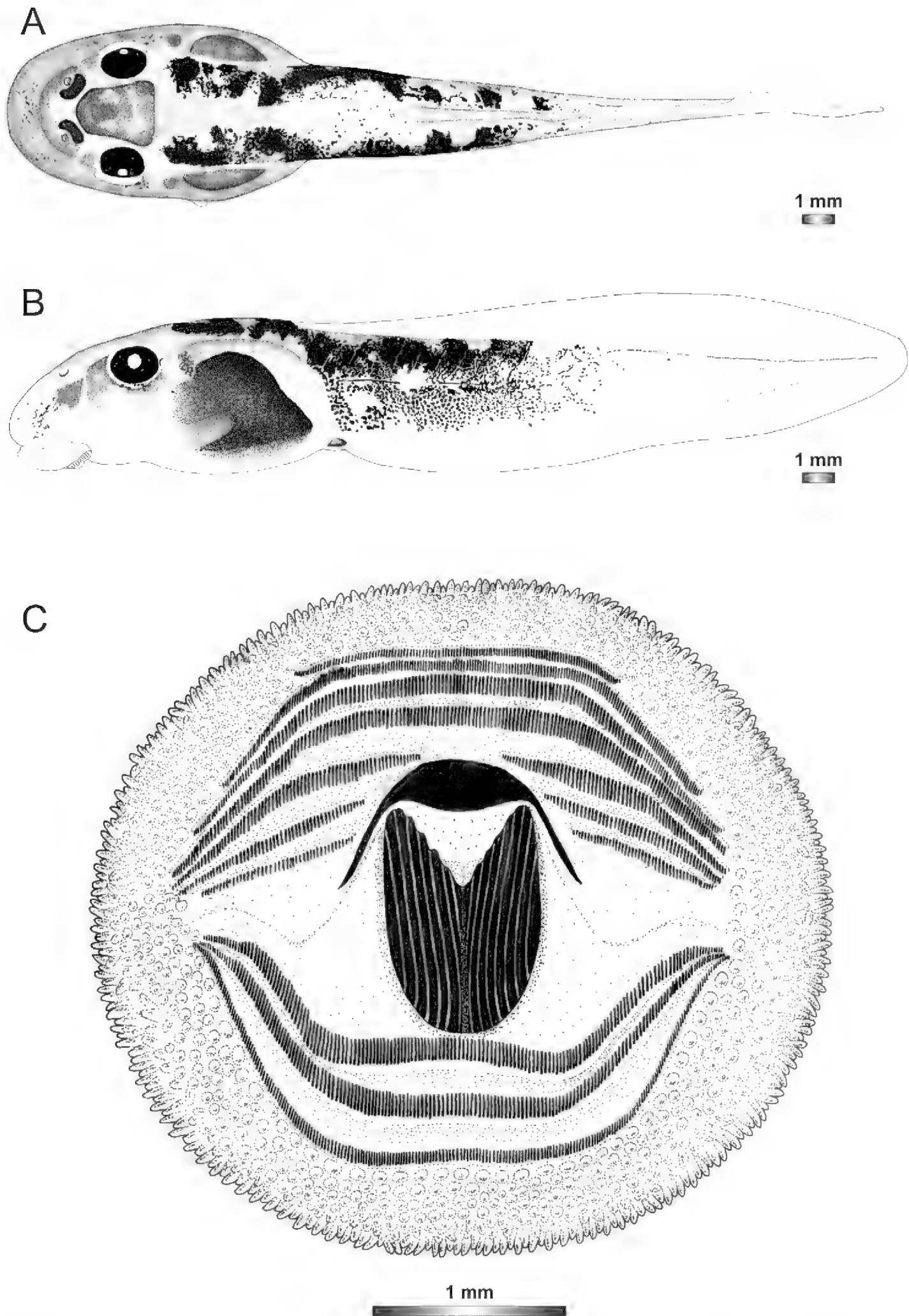
Tadpoles of this candidate species have the typical *marojezensis*-like oral disc structure with 258 marginal and 522 submarginal papillae. These tadpoles are distinguished from other *marojezensis*-like tadpoles by the only poorly recognizable lateral transparent body wall area surrounding the body, and by their tail pigmentation pattern which lacks melanophoric pigments (Figure 1). The position of the eyes is in the range of most other *B. marojezensis*-like tadpoles.

### ***Boophis marojezensis* [Ca53 JQ518216]**

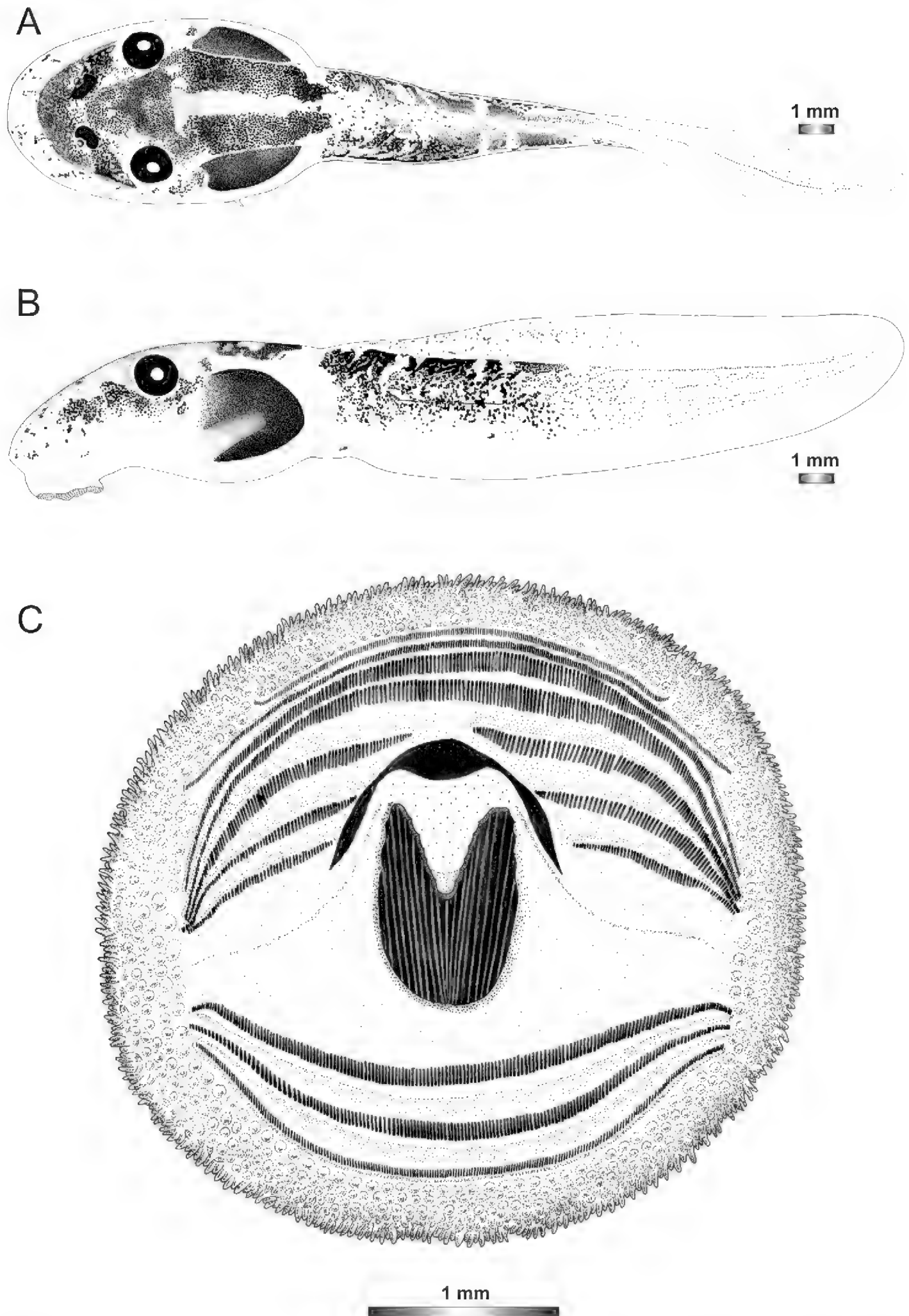
Morphological data were assessed in one tadpole (Figures 2 and 23) in developmental stage 27 (field number ZCMV 13200, ZSM 573/2010, BL 9.6 mm, TL 23 mm, Genbank accession number JQ518216) from Tsaratanana Integral Reserve. The 16S rDNA sequence of this specimen is 98.8% identical to a reference sequence of a *B. marojezensis* adult specimen (accession FJ559127) from Marojejy. Five other voucher specimens attributed to the same candidate species are morphologically very similar to the described one.

The tadpoles of this candidate species have also a *marojezensis*-like oral disc with 243 marginal and 452 submarginal papillae. They are similar to *B. marojezensis*, *B. marojezensis* [Ca25], and *B. marojezensis* [Ca26], but different from *B. marojezensis* [Ca51] and *B.*





**Figure 22.** Drawings of the preserved DNA voucher tadpole of *B. marojezensis* [Ca52] (ZCMV 13168-ZSM 541/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.



**Figure 23.** Drawings of the preserved DNA voucher tadpole of *B. marojejensis* [Ca53] (ZCMV 13200-ZSM 573/2010): **A** Dorsal view **B** Lateral view **C** Oral disc.

*marojezensis* [Ca52] by the presence of a distinct lateral clear area surrounding the body (Figure 1). The general pigmentation pattern is similar to that of *B. marojezensis* [Ca26].

### ***Boophis vittatus* Glaw, Vences, Andreone & Vallan, 2001**

Morphological data were assessed in one tadpole (Figures 2 and 24) in developmental stage 29 (field number FGZC 2238, ZSM 1906/2007, BL 7.8 mm, TL 18.5 mm, Genbank accession number JQ518200), from Marojejy National Park - Camp Mantella. The 16S rDNA sequence of this specimen is 100% identical to a reference sequence of a *B. vittatus* adult specimen (accession FJ559158) from the same locality. Three other voucher tadpoles of *B. vittatus* are morphologically very similar to the described voucher specimen.

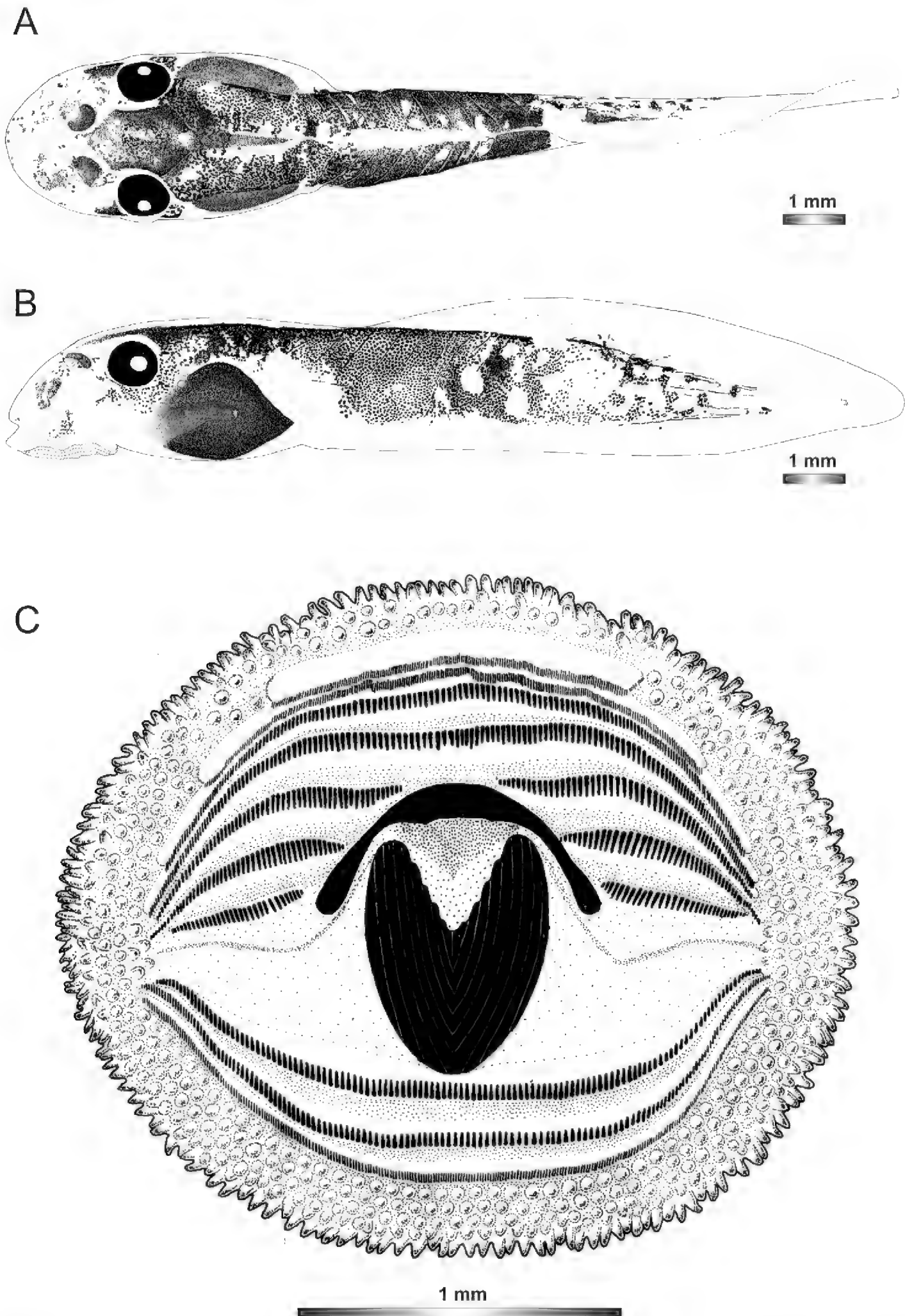
The tadpoles of *B. vittatus* are the smallest tadpoles in this group. They have also a *marojezensis*-like oral disc structure with 289 marginal and 326 submarginal papillae. The tadpoles of this species are provided with a lateral transparent area of the body wall which is more pronounced surrounding the 2/3 anterior of the body. The tail musculature is reticulated like in *B. marojezensis* [Ca51].

### **Occurrence of strongly rheophilous *Boophis* tadpoles in streams of Ranomafana**

In streams of Ranomafana National Park, during the wet season, tadpoles of 44 frog species were found of which five had the morphological characteristics of the strongly rheophilous *Boophis*. These species were found in eleven out of 33 streams. *Boophis andohahela* occurred in eight streams with a mean of 9.9 specimens (min=1 to max=31 specimens), *B. ankaratra* occurred in two streams each with one single specimen, *Boophis marojezensis* [Ca51] was found in seven streams with a mean of 6.3 specimens (1 to 16 specimens), and only a single specimen of *B. schuboeae* was found. The tadpoles of *B. luciae* (named *B. sp. 17* in Vieites et al. 2009) were found in eight streams with a mean of 12 specimens (1 to 33). During the dry season, 23 species were found of which three belong to the group of strongly rheophilous *Boophis*. Those species were found in 30% of the sampled streams in this season. *B. andohahela* occurred in 23% of the streams with nine specimens on average, *Boophis marojezensis* [Ca51] occurred in 30% of the sampled streams with three specimens on average, and *B. luciae* occurred in 15% of the sampled streams with eight specimens on average.

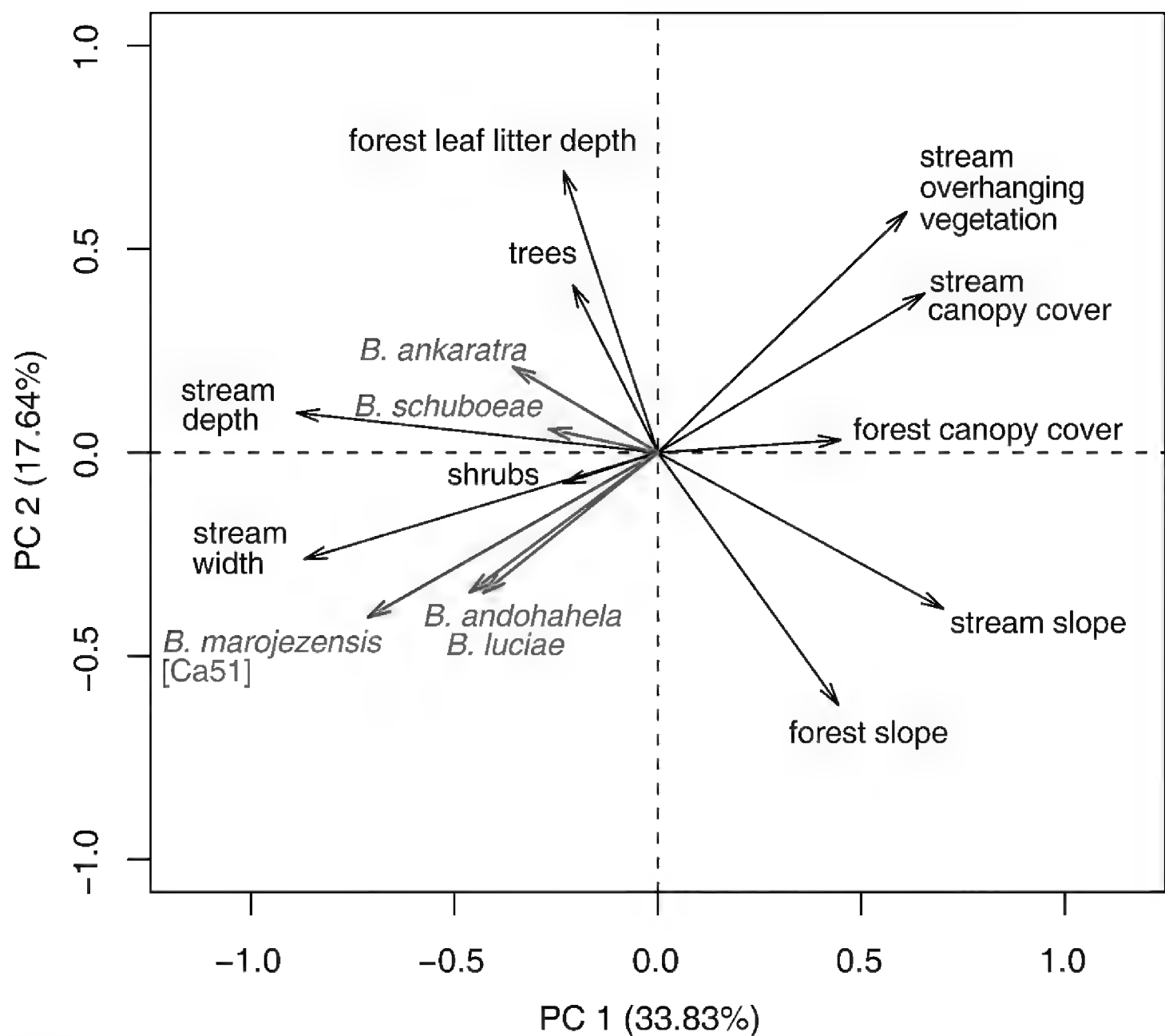
### **Breeding site choice**

Principal Component Analysis on the habitat variables of the stream and the surrounding forest at Ranomafana resulted in three principal components, explaining



**Figure 24.** Drawings of the preserved DNA voucher tadpole of *Boophis vittatus* (FGZC 2238-ZSM 1906/2007): **A** Dorsal view **B** Lateral view **C** Oral disc.

together 65.5% of the variation in the data. We identified the following habitat variables being well represented (Figure 25): PC1 (33.8%) positive: slope and canopy cover of forest and stream, overhanging vegetation; negative: width and depth of the stream. Also four of the strongly rheophilous tadpole species, *B. ankaratra*, *B. andohahela*, *B. luciae*, and *B. marojezensis* [Ca51] are negatively correlated with this PC. The strongest contributors to PC2 (17.6%) were positive: forest leaf litter depth, stream overhanging plants, trees, and stream canopy cover; negative: slope of forest and stream. *B. andohahela* and *Boophis marojezensis* [Ca51] are negatively correlated with this PC. To PC3 (14.1%), the following variables were positive: number of small trees and shrubs in the forest and overhanging vegetation. Due to its rareness, no correlation of *B. schuboeae* incidence and PCs can be statistically assessed.



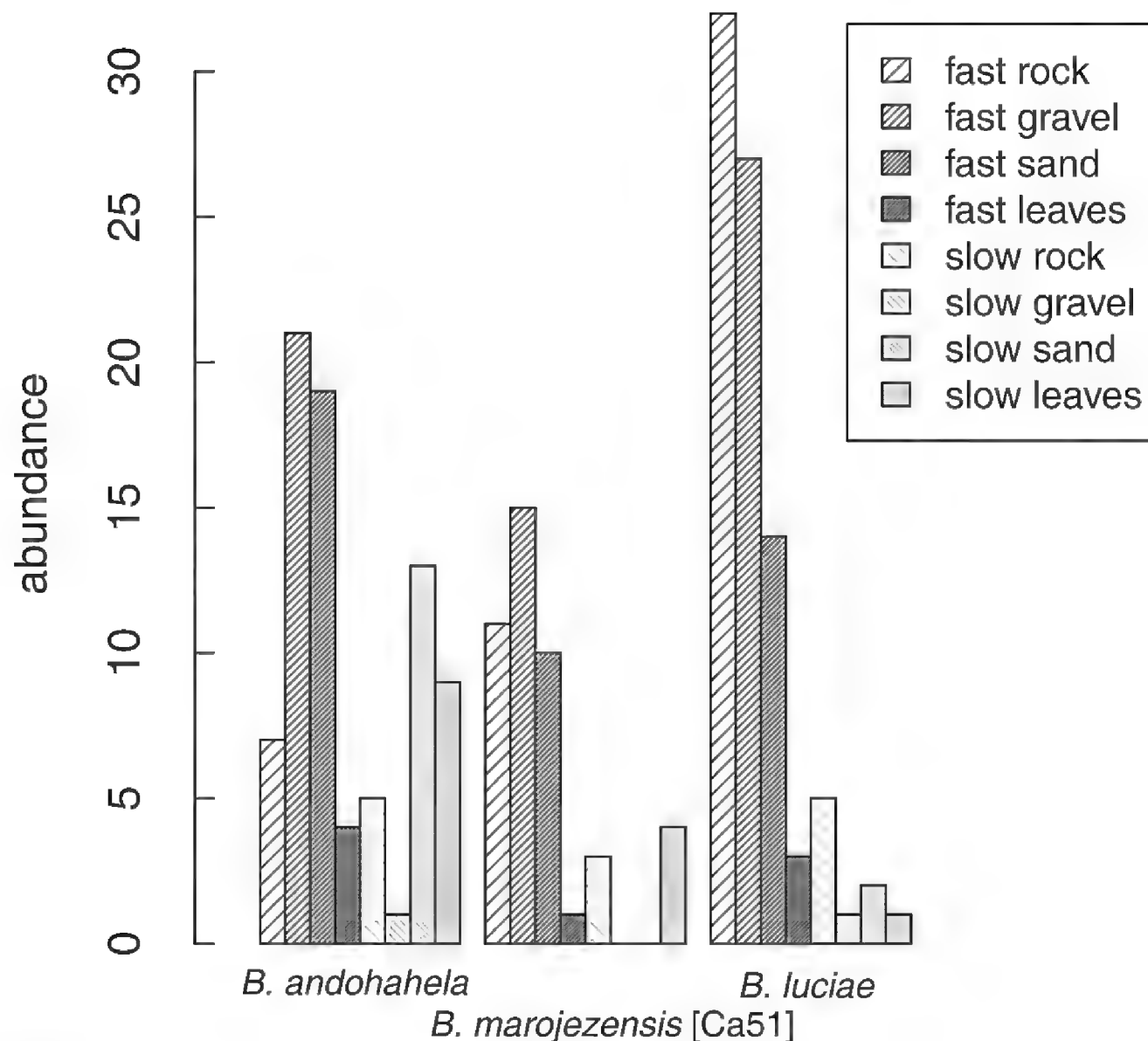
**Figure 25.** PCA biplot of variables of stream and surrounding habitat as recorded during a tadpole community study in Ranomafana National Park. The five present species of strongly rheophilous tadpoles are included as supplementary variables. Length and direction of vectors can be interpreted as correlations.



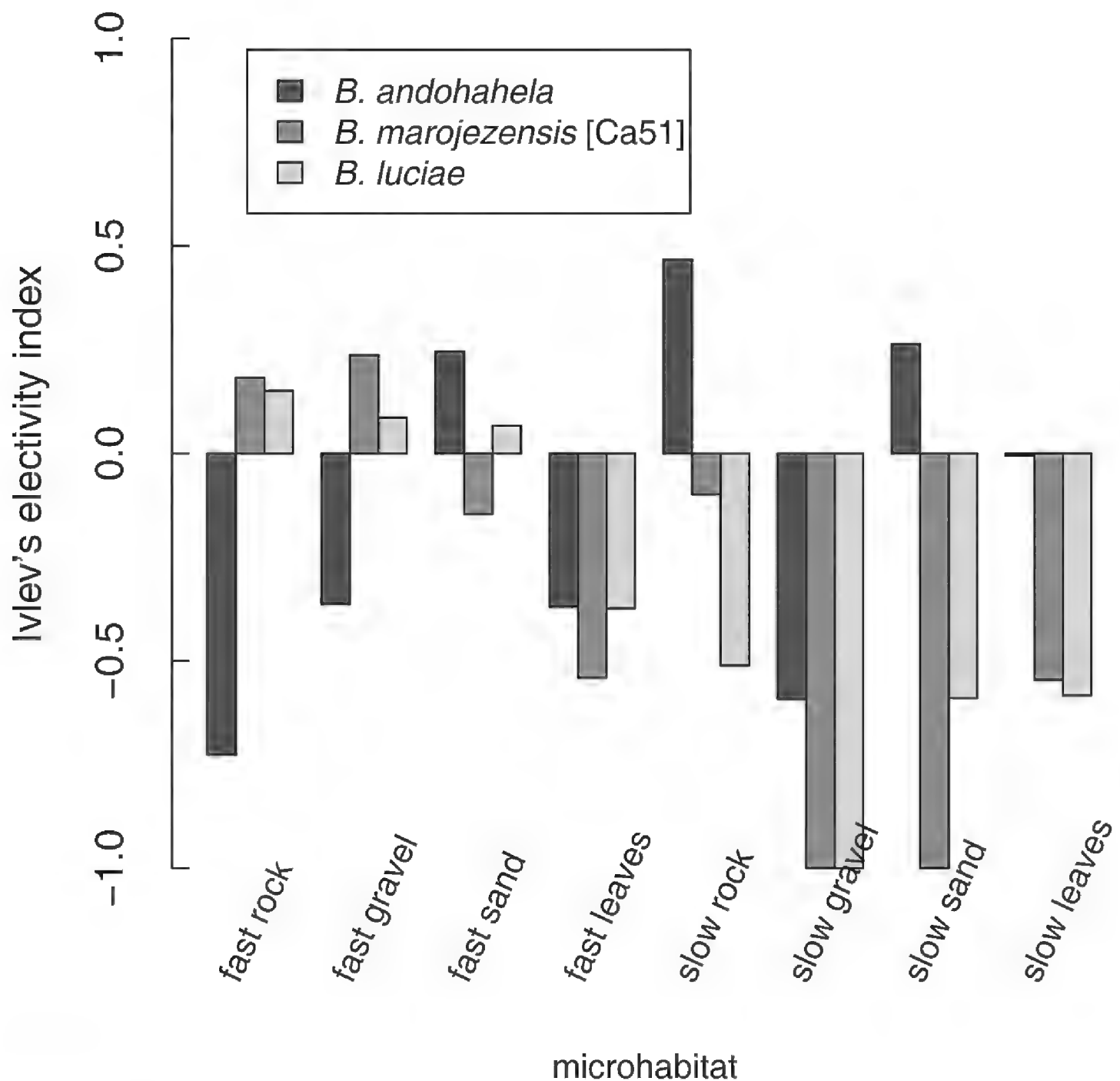
### Microhabitat choice

Strongly rheophilous *Boophis* tadpoles were found in all microhabitats available in streams of Ranomafana National Park (Figure 26). A considerable amount of specimens was found in microhabitats characterised by fast flowing water and substrates of rock, gravel, and sand which generally do not harbour many tadpoles (own unpublished data). Tadpoles of *B. andohahela* were also relatively often found in slow moving parts of the streams with leaves and sand as substrates. Of the two locally rare species, *B. ankaratra* and *B. schuboeae*, one specimen of each was found in fast rock and fast sand microhabitat, and one specimen in slow rock microhabitat, respectively.

Considering the availability of microhabitats in the streams, Ivlev's electivity index (E, Ivlev 1961) shows that strongly rheophilous *Boophis* do not show a consistent microhabitat preference or avoidance except for “slow gravel” which is avoided by all species, and there is no general difference between the three species (Figure 27); factorial ANO-



**Figure 26.** Tadpole distribution across the eight microhabitats (defined using water current and stream substrat) of the three most abundant strongly rheophilous *Boophis* that were sampled in Ranomafana National Park in wet season 2008. *B. andohahela*: N=8 , *B. marojezensis* [Ca51]: N=7, *B. luciae* N=10 with N= the number of streams.



**Figure 27.** Barplot displaying tadpole microhabitat use of three most abundant *Boophis* species with strongly rheophilous tadpole morphology. Microhabitat use is calculated using Ivlev's electivity index (E, Ivlev 1961) with positive values representing microhabitat preferences and negative values representing microhabitat avoidance. For each species, only streams with at least eight specimens were used for analysis (*B. andohahela*: N=3, *B. marojezensis* [Ca51]: N=2, *B. luciae*: N=4 with N= the number of streams).

VA without interaction term including only streams with at least eight specimens of the respective species;  $F_{9,53}=1.716$ ,  $p_{\text{model}}=0.108$ ,  $p_{\text{SG}}=0.008$ , all other  $p$  including the factor “species”  $p>0.26$ ). As interaction terms could not be included in this factorial ANOVA due to overparameterisation, we performed ANOVAs of subsets of the data and found that inter-species differences could only be shown for the microhabitat “fast rock” which is strongly avoided by *B. andohahela* (ANOVA of microhabitat subset;  $F_{2,5}=22.6$ ,  $p_{\text{model}}=0.003$ ,  $p_{B. \text{andohahela}}<0.001$ ) whereas *B. marojezensis* [Ca51] and *B. luciae* were found much more often than *B. andohahela* ( $p_{B. \text{marojezensis} [\text{Ca51}]}=0.003$ ,  $p_{B. \text{luciae}}=0.002$ ). For “slow sand”, only for *B. marojezensis* [Ca51] an avoidance could be detected (ANOVA of microhabitat subset;  $F_{2,5}=3.829$ ,  $p_{\text{model}}=0.098$ ,  $p_{B. \text{marojezensis} [\text{Ca51}]}=0.047$ ), *B. andohahela* and

*B. luciae* used “slow sand” as much as available ( $p_{B. andohahela}=0.427$ ,  $p_{B. luciae}=0.105$ ). For all other microhabitats, no significant difference in microhabitat use of species could be detected. However, it should be noted that missing significances can be caused by the number of replicates (streams) which were reduced as we considered only streams with at least eight specimens of the respective species. A graphical evaluation of microhabitat use indicates that non-preferences or non-avoidances are in fact present (Figure 27).

## Discussion

### Comparisons to previous descriptions of strongly rheophilous *Boophis* tadpoles

Twenty-two strongly rheophilous tadpoles are characterized morphologically in this study, including fourteen tadpoles that are described for the first time and eight other species that had been previously described by other authors. Strongly rheophilous *Boophis* tadpoles have long been known by the work of Blommers-Schlösser (1979), and we here compare her descriptions of *Boophis majori*, *B. sp.*, *B. erythrodactylus*, and *B. mandraka* larvae with current knowledge.

The tadpoles of *B. majori* described by Blommers-Schlösser (1979) correspond to those here assigned to the *B. marojezensis* complex, which is reasonable because also the taxonomic concept of *B. majori* of Blommers-Schlösser (1979) included *B. marojezensis*, a species that was only described later by Glaw et al. (2001). Assigning her *B. majori* tadpoles to one of the *B. marojezensis*-like candidate species is supported by their general external morphology with the sinistral spiracle situated on the 3/4 of the body, the well developed caudal musculature, the dark pattern on the body dorsum, the golden ventral side, the oral disc composed by complete small papillae, the lower jaw sheath that is higher than wide, the presence of an upper jaw sheath, and the LTRF of 7(5–7)/3. However, the finding of a dorsal gap of the papillae in some tadpoles is not in accordance with our description, because all *marojezensis*-like tadpoles (*B. marojezensis*, *B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*) lack a dorsal gap of papillae (data herein and in Raharivololoniaina et al. 2006). We conclude therefore that those tadpoles mentioned by Blommers-Schlösser (1979) might be strongly rheophilous tadpoles from another species, possibly belonging to the *B. albipunctatus* group. Additionally, the relative tail length which is two times of the body length and the situation of the nares close to the eyes of the tadpoles examined by Blommers-Schlösser (1979) do not agree with our data, because all relevant tadpoles in this study have a rather short tail (TAL 166 – 188% of BL) and an opening of the nares that is closer to the snout than to the eyes or in the middle (RN/NP 78 – 103%).

The tadpoles of *Boophis* sp. (Blommers-Schlösser, 1979) are similar to the *B. luteus* group tadpoles described herein according to their general oral disc structure. The LTRF 6(3–6)/3(1) corresponds to those of *B. englaenderi* tadpoles and 6(3–6)/3 to those of *B. englaenderi* [Ca23] tadpoles: This indicates that these tadpoles might be-

long to two different *Boophis* species. Since *B. englaenderi* and *B. englaenderi* [Ca23] do not occur in the site where Blommers-Schlösser (1979) observed her *Boophis* sp. tadpoles, we hypothesize that those tadpoles belong to species in *B. luteus* group whose larval stages are not yet known.

Tadpoles having narial openings closer to the eyes than to the snout, a sinistral spiracle situated on the  $3/4$  of the body, a well developed caudal musculature, a rounded oral disc with a LTRF of  $7(5-7)/3$ , a dorsal gap of papillae and a complete jaw sheath were also described and assigned to *Boophis erythrodactylus*, a species of the *B. rappiodes* group, by Blommers-Schlösser (1979). The species identification of those tadpoles, however, is uncertain as already mentioned by Raharivololoniaina et al. (2006): (1) all the other species of the *B. rappiodes* group have generalized tadpoles, i.e., *B. rappiodes* as described in Blommers-Schlösser (1979) and Raharivololoniaina et al. (2006), *B. tasymena* and *B. viridis* in Raharivololoniaina et al. (2006), and *B. bottae* in Randrianiana et al. (2009a), and (2) those tadpoles were stated to occur in sympatry with *B. mandraka* tadpoles, and either might just be a variation of *B. mandraka* tadpoles or belong to a closely related species of *B. mandraka* with similar tadpoles. As we encountered several times in our study, the tadpoles of two closely relative species can live sympatrically.

As described by Blommers-Schlösser (1979), the tadpoles of *B. mandraka* have a sinistral spiracle that opens at  $2/3$  of the body, narial openings closer to the eyes than to the snout, a tail that is two times longer than the body, a well developed caudal musculature, a silvery belly, an almost rounded oral disc with a V-shaped lower sheath and a LTRF of  $7(6-7)/3$ . So far no strongly rheophilous tadpoles with only two interrupted upper keratodont rows have been observed in our study. We have observed in some tadpoles of *B. sibilans* and *B. luciae* that the gap separating the  $A_5$  row is very tight which might be responsible for the false impression of an uninterrupted row.

Tadpoles of *B. andohahela* from Ranomafana were described by Thomas et al. (2006). The general morphology and the oral disc structure of the tadpoles agrees with our specimens, except the keratodont row formula and the presence of a ventral gap of marginal papillae. Thomas et al. (2006) described tadpoles with a LTRF  $6(3-6)/3(1)$ , although in our study all tadpoles from the same locality as in Thomas et al. (2006) have a LTRF  $6(3-6)/3$ . This might be caused by the fact that the teeth in the first lower row are very dense, and sometimes it folds in the middle giving the mistaking impression of a gap.

The *B. sibilans* tadpoles from Andasibe that Raharivololoniaina et al. (2006) described agree with our specimen except some minor differences; e.g., the relative width of the oral disc. These differences might be due to the different developmental stages of the tadpoles in the two studies, or by the different methods that have been used for taking the respective measurements.

Glos et al. (2007) described the tadpoles of *B. schuboeae* from Ranomafana and of *B. ankaratra* from Andringitra. The morphology therein is in accordance to the specimens of our study.

*B. englaenderi*, *B. vittatus* and *B. luciae* were described by Rasolonjatovo et al. (2010). We redescribe these species because of the bad condition of the voucher speci-

mens and/or the lack of some data in the previous descriptions. The same tadpole specimen of *B. englaenderi* from Marojejy National Park was redescribed to facilitate the comparison to the other *B. luteus* group tadpoles. We furthermore described the tadpoles of *B. vittatus* and *B. luciae* from the same locality based on new voucher specimens because of the bad condition of the vouchers used in Rasolonjatovo et al. (2010).

### Morphological differences among tadpoles of closely related species

As described by Blommers-Schlösser (1979), defined by Raharivololoniaina et al. (2006), confirmed by Glos et al. (2007) and observed herein, strongly rheophilous tadpoles are typical stream-inhabiting organisms, and are characterized by a narrow and flat elongated body, a well developed caudal musculature, a wide oral disc with many small papillae that are either complete or interrupted by a dorsal gap, a rather small and ribbed (i.e., composed of a series of fused columns) lower jaw sheath, many upper keratodont rows with at least the two first being uninterrupted and three lower keratodont rows of which in most of the species the first one is uninterrupted.

This type of tadpoles can be found in different *Boophis* species groups: *B. luteus* group, *B. albipunctatus* group, *B. mandraka* group, and *B. majori* group (its occurrence in the *B. rappiodes* group is in need of confirmation). As described by Blommers-Schlösser (1979) and Schmidt et al. (2008), also *B. williamsi* (*B. microtypanum* group) has an enlarged oral disc (ODW 90% of BW, pers obs.) with a LTRF of 8(3–8)/3. However, we did not consider this species in our study because (1) this tadpole has a generalized oral disc structure (jaw sheaths, papillae and keratodonts) and (2) all the other strongly rheophilous tadpoles have a rather small size (BL 5.9 – 13.5 mm, TL 12.7 – 27.1 mm, in Gosner stages 25 – 36) compared to the montane *B. williamsi* tadpoles (BL 25.5 mm and TL 71.7 mm in Gosner stage 36).

Within the main groups of morphologically similar tadpoles, some can be very similar, but usually there are morphological details to differentiate them, whether in the external morphology or in the oral disc configuration; i.e., tadpoles that are very similar in external morphology can be differentiated in oral disc structure and vice versa:

- (1) Three tadpoles belonging to the *Boophis luteus* group (*B. englaenderi*, *B. englaenderi* [Ca23], and *B. andohahela*) look alike in external morphology but can be differentiated easily by their keratodont row formula. Of these, *B. englaenderi* and *B. englaenderi* [Ca23] occur syntopically. The tadpoles of *B. englaenderi* [Ca23] can be distinguished from those of *B. englaenderi* by their relative tail length, by their pigmentation pattern and mainly by their oral disc structure (LTRF and number of papillae).
- (2) In the *B. albipunctatus* group, *B. ankaratra*, *B. schuboeae*, *B. sibilans* and *B. luciae* are similar. *B. ankaratra* and *B. schuboeae* occur sympatrically, and they can be differentiated by the presence of dark pigmented bands on the tail muscle in *B.*



*schuboeae*, and also by the absence of papillae on the lateral area where the oral disc folds in *B. schuboeae*. *B. sibilans* and *B. luciae* differ by the presence of a dark bridge which connects the dark sections on the tail muscle in *B. luciae*.

- (3) All tadpoles known from the species of the *B. mandraka* group have a similar oral disc configuration, characterized by the absence of the upper jaw sheath and a LTRF of 8(6–8)/3. The tadpoles of *B. sambirano* and *B. mandraka* [Ca46] are very similar, except that *B. mandraka* [Ca46] has the narrowest dorsal gap of marginal papillae. The fact that these two tadpoles live allopatrically can help also to identify them. Five species of this group are distributed in close proximity in the North of Madagascar. Of these, *B. sambirano* [Ca47] and *B. sambirano* [Ca48] are sympatric, and can be differentiated by the patched *vs.* spotted pattern on the tail. *B. sambirano* [Ca49] and *B. sambirano* [Ca50] live also sympatrically. *B. sambirano* [Ca49] can be distinguished to the three other species by its generally dark coloration pattern, the ovoidal form of the body in dorsal view and the wide inter-orbital distance. *B. sambirano* [Ca50] can be differentiated by the intensity of the golden pigments which may cover the whole body and overlay the dark pigment in some specimens. *B. mandraka* [Ca38] is very typical by its weakly expressed pigmentation.
- (4) Two tadpoles belonging to two different groups, *B. albipuncatus* (*B. albipuncatus* group) and *B. mandraka* [Ca38] (*B. mandraka* group) are similar in their weak expression of pigmentation, but they can easily differentiated by their oral disc morphology.
- (5) Two cases of similarity are also found in *B. majori* group tadpoles. *B. marojezensis*, *B. marojezensis* [Ca26], *B. marojezensis* [Ca53] and *B. vittatus* are very similar in the presence of a clear, not pigmented lateral area surrounding the body, and in the tail pigmentation pattern. The fact that several of these species can occur sympatrically increases also the chance to confound them. On the other hand, the tadpoles of *B. marojezensis* [Ca51] and *B. marojezensis* [Ca52] are similar in the absence of a lateral clear area surrounding the body, and in their general pigmentation pattern. Only the tadpole of *B. marojezensis* [Ca25] is easily distinguishable by the presence of clear and more or less rounded patches on the tail muscle. As the three *B. marojezensis*-like tadpoles, *B. marojezensis*, *B. marojezensis* [Ca25] and *B. marojezensis* [Ca26], live syntopically in Marojejy National Park, *B. marojezensis* [Ca25] tadpoles will not be confounded with those of the two other species.

### Morphological clusters of strongly rheophilous *Boophis* tadpoles

Analyzing the structure of the oral disc of all these tadpoles allows classifying them into three clusters:

- (1) The first cluster including three *B. luteus* group tadpoles is characterized by a moderately wide to very wide (ODW 56 to 84% of BW), non emarginated,

ventrally positioned and oriented oral disc, which has an anterior margin connected directly to the snout, two uninterrupted upper rows of keratodonts (LTRF is 6(3–6)/3(1) for *B. englaenderi* but 6(3–6)/3 for the tadpoles of *B. englaenderi* [Ca23] and *B. andohahela*); a very long  $A_1$  (82 to 90% of ODW); a high number of keratodonts in  $A_1$  (220 to 301), totally keratinized; typically narrow to moderate sized jaw sheaths (JW 31 to 46% of ODW) with a very short medial convexity (MCL 0.04 to 0.11% of JW); a wide to very wide dorsal gap of papillae (DG 67 to 85% of BW); a low number of submarginal papillae (33 to 94) and a medium number of marginal papillae (101 to 175); a high positioned eye (EH 69 to 85% of BH) that is situated not far from midbody (SE 32 to 39% of BL); very high positioned nares (NH 57 to 82% of BH) that are situated below or at eye level (NH 82 to 97% EH) and closer to the snout than to the eye (RN 60 to 92% of NP); a short tail (TAL 155 to 183% of BL), and a developed caudal musculature.

- (2) The second cluster is characterized by a wide to hyper-wide (ODW 74 to 108% of BW) non emarginated, ventrally positioned and oriented oral disc with an anterior margin separated from the snout by a shallow crevice or free; four or five uninterrupted upper rows of keratodonts giving a LTRF of 7(5–7)/3 or 8(6–8)/3; a short to moderately sized  $A_1$  (21 to 59% of ODW); a low to medium number of keratodonts in  $A_1$  (95 to 241), totally keratinized; U-shaped, ribbed narrow upper jaw sheaths (JW 30 to 34% of ODW which can present a small medial convexity or not); a U-shaped and "ribbed" lower sheath, a moderately wide to very narrow dorsal gap of papillae (DG 14 to 59% of BW); a medium to high number of marginal papillae (148 to 377), many submarginal papillae (190 to 368); high to very high positioned eyes (EH 71 to 84% of BH) that are situated closer to the snout than to midbody (SE 35 to 49% of BL), high to very high positioned nares (NH 64 to 92% of BH) that are situated below or above the eye level (NH 86 to 112% of EH) and closer to the eye than to the snout (RN 107 to 194% of NP); a short to very short tail (TAL 146 to 184% of BL); and a developed caudal musculature. Tadpoles of the *B. albipunctatus* group (*B. schuboeae*, *B. ankaratra*, *B. albipunctatus*, *B. sibilans*, and *B. luciae*) and *B. mandraka* group (*B. sambirano*, *B. mandraka* [Ca38], *B. mandraka* [Ca46], *B. sambirano* [Ca47], *B. sambirano* [Ca48], *B. sambirano* [Ca49], and *B. sambirano* [Ca50]) belong to this cluster. All *B. mandraka* group tadpoles lack a keratinized upper jaw sheath.
- (3) The third cluster is characterized by a wide (ODW 68 to 79% of BW) non emarginated, ventrally positioned and oriented oral disc without a dorsal gap of papillae and with the anterior margin being free from the snout; four uninterrupted upper keratodont rows (LTRF 7(5–7)/3); a moderately sized  $A_1$  (45 to 52% of ODW); a medium number of keratodonts in  $A_1$  (126 to 235); a totally keratinized upper jaw sheath (JW 30 to 38% of ODW) without medial convexity; a U-shaped and ribbed lower sheath, many submarginal (222 to 318) and marginal

(206 to 522) papillae; high positioned eyes (EH 68 to 80% of BH) that are situated closer to midbody (SE 35 to 49% of BL); very high positioned nares (NH 68 to 80% of BH) that are situated below eye level except for *B. vittatus* and *B. marojezensis* [Ca25] (NH 89 to 101% of EH) and closer to the snout for the most (RN 78 to 109 % of NP); a very short to short tail (TAL 140 to 188% of BL), and a developed caudal musculature. Tadpoles of the *B. majori* group (*B. marojezensis*, *B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*) belong to this cluster.

Although we lack at this time an explicit and well supported phylogenetic hypothesis for the relationships among all these species of *Boophis*, the morphological characters of these three morphological clusters can serve to develop a possible evolutionary scenario of the origin of the specializations in strongly rheophilous *Boophis* tadpoles, departing from the structures in more generalized *Boophis* tadpoles. All of the latter are characterized by having one (the first) uninterrupted upper keratodont row and one (the first) interrupted lower keratodont row, typically smooth (non-ribbed) jaw sheaths and a medial convexity in the upper jaw sheath (see Raharivololoniaina et al. 2006, Randrianiana et al. 2009a, b, and Rasolonjatovo et al. 2010). Specialization to a strongly rheophilous life thus involves (1) reduction of the size of the jaw sheaths correlated with (2) the disappearance of the medial convexity, (3) reduction of the size of the dorsal gap of marginal papillae, (4) reduction of the length of the row  $A_1$ , (5) reduction of the number of keratodonts in  $A_1$ , compensated by an increase of the number of (6) marginal and (7) submarginal papillae and (8) of the uninterrupted upper keratodont rows.

The decrease of the size of the jaw sheaths may provoke the fading of its medial convexity on one hand and leaves a place for many dorsal and lateral, even ventral submarginal papillae, and new uninterrupted upper keratodont rows on the other hand. Also, the reduction of the size of the dorsal gap leads to a higher number of marginal papillae. The development of many dorsal marginal papillae reduces the area available for the first upper keratodont row and thus may cause the reduction of its length, which in turn leads to the decrease of the number of the teeth. However, the loss of the upper jaw sheath in all species and candidate species of the *B. sambirano* complex is still unclear. This characteristic is neither caused by a fixation artifact nor by the transportation of the specimens because we observed it already in the living tadpoles in the field (Figure 29), and because it is consistent within series. The absence of the upper jaw sheath was found even in young tadpoles (Gosner 25) indicating that it occurs very early in larval development. It remains to be tested (e.g., by a study on embryonic development), however, if this structure never develops, or is initially formed but then disappears at some early developmental stage.

### Ecomorphological guilds in *Boophis* tadpoles

A magnitude of descriptions of the larval stages of Madagascan frogs have been recently published (Andreone et al. 2002, Glos and Linsenmair 2005, Raharivololoniaina et

al. 2003, 2006, Thomas et al. 2005, 2006, Altig and McDiarmid 2006, Vejarano et al. 2006a, b, c, Glos et al. 2007, Schmidt et al. 2008, 2009a, b, Grosjean and Vences 2009, Jovanovic et al. 2009, Rasolonjatovo et al. 2010, Grosjean et al. 2006, 2007, 2011a, b, Randrianiaina et al. 2007, 2009a, b, 2011a, b, c). While some of them merely intended to increase general knowledge on Madagascan tadpoles, others attempted to classify the tadpoles into ecomorphological guilds. For *Boophis* tadpoles, Raharivoloniaina et al. (2006) tried to define three guilds, named A, B and C, mainly based on three variables: relative width of oral disc, number of inframarginal papillae, and number of keratodonts on the first anterior row. As already mentioned by Randrianiaina et al. (2009a), these guilds were not intended to replace nor to refine the guilds of Altig and Johnston (1989), but to achieve a complementary, more quantitative classification that would better fit the variation of the *Boophis* tadpoles studied. Moreover, the criteria chosen by Raharivoloniaina et al. (2006) were few and some of those that Altig and Johnston (1989) used do not exist in *Boophis* tadpoles (Randrianiaina et al. 2009a). Therefore, a comprehensive definition of adequate guilds for Malagasy tadpoles will require the consideration of numerous new variables without omitting those that have been used before. In this process it is important to notice first the presence or absence of one component (e.g., jaw sheath and keratodont) and then its configuration (e.g., totally or poorly keratinized sheaths, density of papillae; Randrianiaina et al. 2011a).

According to Altig and Johnston (1989), three different guilds might correspond to *Boophis* tadpoles. The clasping tadpoles have a dorsal gap of marginal papillae, commonly five keratodont rows (but as numerous as 8/8), usually with anterior rows that are more numerous than posterior rows (e.g., 9/3), and a globular to slightly depressed body. They inhabit medium to slow water currents and the maintenance of their position in the water current with the help of the oral disc is of minor importance. The adherent tadpoles have small and complete marginal papillae, and a LTRF of commonly 2/3. They inhabit faster flowing water than clasping tadpoles, their position maintenance via the oral disc is common to continuous, and their body is often depressed. The suctional tadpoles have a depressed body, small and complete marginal papillae, and a LTRF from 2/3 to a maximum of 17/21. They inhabit even faster running waters than the clasping and adherent tadpoles, and their position maintenance via their oral disc is continuous.

In this study, no new guild names are defined, but we suggest to adapt in a preliminary way the guilds already defined by Altig and Johnston (1989).

- (1) We do not consider the *B. luteus* group tadpoles truly strongly rheophilous, due to their more generalized and intermediate characteristics. These tadpoles (*B. englaenderi*, *B. englaenderi* [Ca23], *B. andohahela*) can possibly be considered to be part of the “clasping” guild.
- (2) The first guild of strongly rheophilous tadpoles, here considered as “adherent”, is the second category of tadpoles classified in the previous section which is composed by the tadpoles of the *B. albipunctatus* group (*B. schuboeae*, *B. ankaratra*, *B. albipunctatus*, *B. sibilans*, and *B. luciae*) and the *B. mandraka* group

- (*B. sambirano*, *B. mandraka* [Ca38], *B. mandraka* [Ca46], *B. sambirano* [Ca47], *B. sambirano* [Ca48], *B. sambirano* [Ca49], and *B. sambirano* [Ca50]), because they inhabit faster running water and the maintenance of the position in the water via their oral disc is common to continuous. This guild is characterized mainly by the presence of a dorsal gap of papillae and two typical LTRF-s which are  $8(5-8)/3$  and  $8(6-8)/3$ . All *B. mandraka* group tadpoles lack an upper jaw sheath, while this structure is present in the *B. albipunctatus* group tadpoles.
- (3) The second guild that we define as “suctorial” is the third category of tadpoles classified in the previous section which is composed of all *B. marojezensis*-like tadpoles (*B. marojezensis*, *B. marojezensis* [Ca25], *B. marojezensis* [Ca26], *B. marojezensis* [Ca51], *B. marojezensis* [Ca52], *B. marojezensis* [Ca53], and *B. vittatus*). They probably inhabit faster running water and maintain continuously their position in the water with the help of their oral disc because of the complete state of the papillae that they have. This guild is characterized by the absence of a dorsal gap of papillae and a LTRF of  $7(5-7)/3$ .

### Habitat selection and ecology of strongly rheophilous *Boophis* tadpoles

In the tropical rainforest of Ranomafana National Park, strongly rheophilous *Boophis* tadpoles occur throughout the whole year (own unpublished data) with clearly higher abundances in the wet season. Whereas some species are relatively common (e.g., *B. marojezensis* and *B. luciae*), others are locally extremely rare (e.g., *B. ankaratra*, *B. schuboeae*). In this area, strongly rheophilous *Boophis* do neither include the most common tadpoles species nor is the group itself as common as other groups (Grosjean et al. 2011). Species of this group choose larger, open, slowly running streams for breeding (Figure 28); small streams with high slope and a dense vegetation cover are generally avoided. This is generally true for all strongly rheophilous species studied in Ranomafana National Park. The latter kind of stream might be avoided as they are less attractive to adults than large streams, which provide more space without the risk of egg and tadpole predation by fishes. Small streams might also be characterised by reduced food availability, e.g., due to reduced periphyton growth as a result of high vegetation coverage (Mallory and Richardson 2005; Altig et al. 2007). This actually describes the expected pattern for most tadpoles in Madagascan rainforest streams and can also be observed, e.g., for tadpoles of the *Mantidactylus* subgenus *Ochthomantis*, which are characterised by reduced oral disc structures (Randrianiana et al. 2011a). In contrast, the also specialized funnel mouthed tadpoles of *Mantidactylus* subgenus *Chonomantis* do not follow this pattern, as for some species no prediction of occurrence by habitat characteristics is possible and some species (e.g., *Mantidactylus opiparis*) prefer combinations of habitat characteristics that are unfavourably represented in our PCs (Grosjean et al. 2011a).

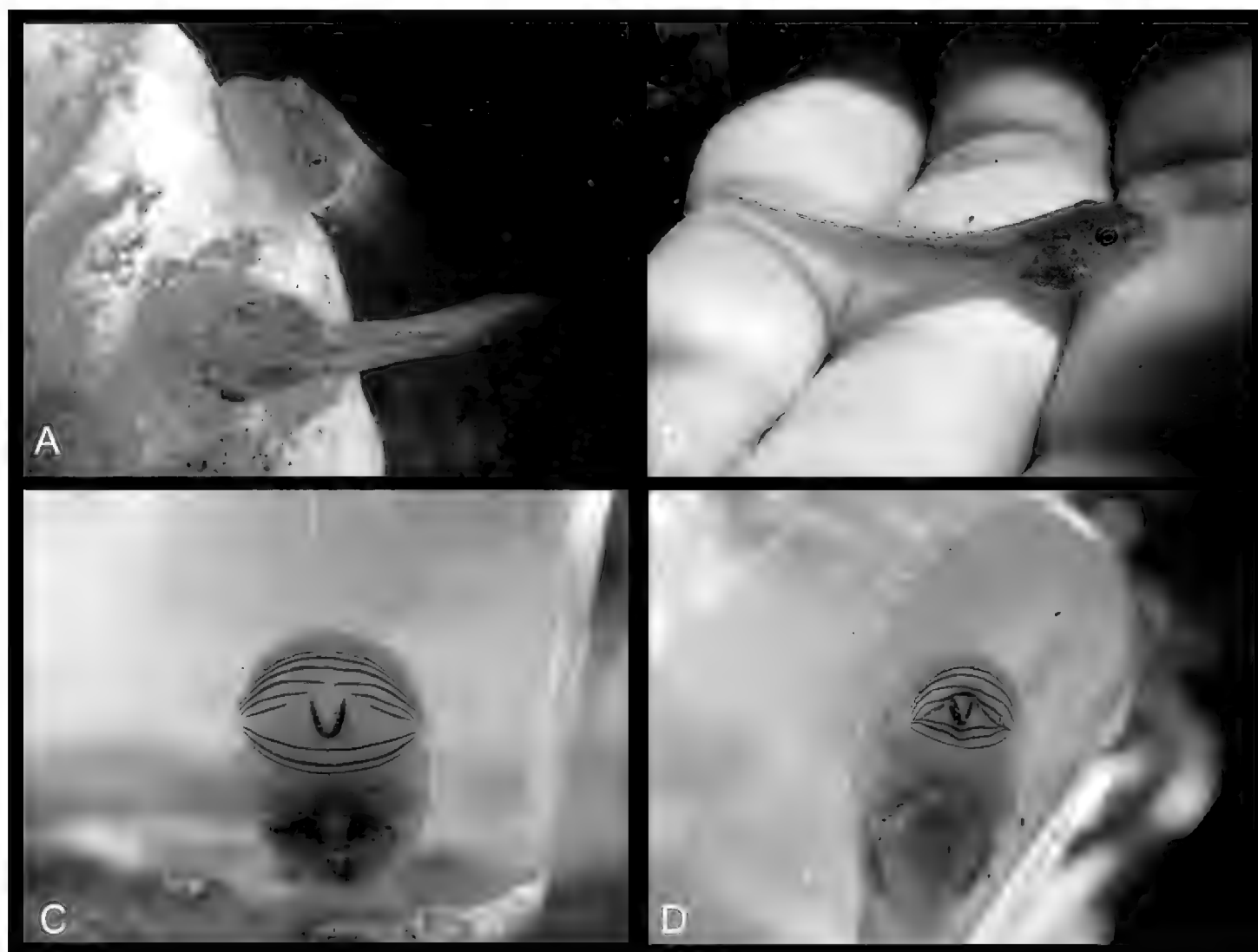




**Figure 28.** Pictures showing tadpole capture sites inside a primary forest in Ranomafana National Park (**A** in Fompohonina river, **B** in Piste E 100 stream), and outside the forest (**C** in Anjingo river and **D** in Ankijagna Lagnana).

Within the streams, however, strongly rheophilous *Boophis* tadpoles are quite outstanding regarding their microhabitat choice compared to other abundant and well observed tadpole groups. This is especially true for two of the most common of these species, *B. marojejensis* [Ca51] and *B. luciae*, and less pronounced for *B. andohahela*, consistent with the more generalized oral disc structure of this latter species. Whereas we could not show true preferences for fast running sections, we could at least show that a considerable number of specimens are indeed using these faster parts of the streams. This clearly separates these tadpoles from other abundant groups (Grosjean et al. 2011a, Randrianiana et al. 2011a), and most likely reflects the morphological specialisations of oral disc, body, and tail to withstand the current.

Their large ventral oral disc allows attaching on substrate (Figure 29) such as rocks and gravel, and the presence of numerous short papillae presumably aids in forming a tight seal between the oral disc and the irregularities of substrate (Altig and McDiarmid 1999). Also, their relatively small body size and well developed caudal musculature probably allows a good locomotory performance in strong current.



**Figure 29.** Photographs in life of strongly rheophilous *Boophis* tadpoles: **A** and **B** Underwater pictures of *B. sambirano*-like tadpoles (from Antevialambazaha - Tsaratanana Integral Reserve) **C** and **D** Oral disc of *B. sambirano* [Ca48] (ZCMV 13109 -ZSM 485/2010) and *B. marojezensis* [Ca52] (ZCMV 13169-ZSM 542/2010) (from Ambinanitelo) fixing on the sides of an aquarium.

### Reverse taxonomy and high cryptic species diversity of *Boophis*

As already demonstrated by Randrianiana et al. (2011a), reverse taxonomy, initially defined for unicellular organisms and invertebrates, can also be applied to better studied groups such as vertebrates. Herein we confirm the usefulness of this method by finding numerous divergent tadpole DNA sequences. Twelve candidate species are defined in this study by the divergent DNA sequence of the tadpoles in comparison with the sequences of all species and candidate species previously known by adult specimens. To evaluate the status of such genetically divergent specimens, it is important to evaluate whether (1) the genetic divergence is correlated with other characters, e.g., consistent morphological differences, and (2) whether these consistently differentiated groups may furthermore occur in sympatry, which then suggests they are reproductively isolated evolutionary lineages, and thus, distinct species. We could indeed find such a situation in three pairs of species, and thus can flag several of the newly discovered genealogical lineages as confirmed candidate species (Vieites et al. 2009):

- (1) *B. englaenderi* [Ca23] lives syntopically with *B. englaenderi*, and these two forms show clear and constant differences genetically and in larval morphology, as described above, including characters of the oral disc, relative tail length, and coloration.
- (2) In the *B. mandraka* group, *B. sambirano* [Ca49] tadpoles are very deviant and can easily be differentiated by coloration and the position of the eyes from the lineage *B. sambirano* [Ca50] occurring at a nearby locality in the same stream.
- (3) *B. marojezensis* [Ca25] is very distinct by the presence of more or less rounded patches on the posterior half of the tail musculature which distinguishes it from the two syntopic forms, *B. marojezensis* and *B. marojezensis* [Ca26].

As a conclusion, this extraordinary and surprising diversity of *B. marojezensis*-like and *B. sambirano*-like candidate species especially in northern Madagascar probably indeed reflects a high number of yet undescribed species, and claims for a biogeographic and evolutionary explanation. It further confirms that stream-breeding frogs apparently show a higher geographical structuring of their diversity (e.g., Inger et al. 1974; Vences et al. 2002). An in-depth revision of these frogs is necessary to understand this diversity and its taxonomic relevance, and needs to be based on an integrative approach assessing their bioacoustic, and nuclear genetic divergence, focusing on sympatric occurrences which we expect to be particularly informative regarding the isolation mechanisms between these lineages.

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## Appendix I

Table 3–5. (doi: 10.3897/zookeys.178.1410.app1) File format: Excel spreadsheet (xls).

**Explanation note:** Appendix I contains Table 3–5.

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## Appendix 2

Morphological description of the rheophilous *Boophis* tadpoles. (doi: 10.3897/zookeys.178.1410.app2) File format: MS Word document (.doc).

**Explanation note:** Appendix II contains complete morphological description of the rheophilous *Boophis* tadpoles.

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